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フォーラム

自主臨床試験に参加した被験者を対象としたCRCの貢献に関する質問票調査

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1．はじめに

医療の質を評価する指標の1つとして患者の視点に立った満足度が重視されるようになった[1,2]．この評価手法は企業主導で行われる新規医薬品や医療機器の開発に関わる臨床試験(以下，治験とする)における被験者の満足度調査にも用いられている[3~7]．近年，臨床試験の質を担保するとともに被験者の満足度を高める観点から治験コーディネーター(CRC)[註]育成が行われ，今やCRCは臨床試験に不可欠な存在となりつつある．治験におけるCRCの有用性は医師および患者を対象とした調査で好意的な評価が得られている[8~10]．

京都大学では2001年に医学部附属病院探索医療センターが発足し，企業主導の治験以外の自主臨床試験においてもCRCが配置され，試験の支援を実施してきた実績がある．そこで，本研究では，自主臨床試験において被験者が試験への参加とCRCの自主臨床支援活動をどのように評価しているか，とくに該当臨床試験参加の動機付けのうえでCRCがどのような関与をしているかを明らかにすることを目的として，当センターで実施した整形外科疾患に対する自主臨床試験[11]の被験者に対して質問票調査法により調査を行ったので報告する．

2．対　象

質問票の対象者は2005年4月から2007年8月までに当センターで行った整形外科領域の第Ⅱ相ランダム化プラセボ対照比較臨床試験[11]に参加した32名〔平均年齢：61歳（44~80歳），男女比：男性7名，女性25名〕であった．治療対象疾患は，変形性股関節症で，治療内容は人工股関節置換術であった．今回の臨床試験では担当医師が病気についての説明や臨床試験の目的や意義などを試験に参加する患者に説明した後に，CRCが別室で臨床試験の詳細なスケジュールや経費，入院生活等やその他の患者の疑問に対して40~60分程度対面形式で説明を行った．CRCによる臨床試験支援についての患者評価は該当試験期間の終了後に郵送による質問票（無記名，選択・記述式，A3両面印刷）で実施した．

3．方　法

【質問票】

質問票の調査項目はTableに示すように試験参加の理由，CRCへの満足度，被験者がCRCに求めるものなど幅広く設定され，CRCの臨床試験への貢献内容を多面的に調査できるよう計画された．調査項目に対しては原則として5段階評価での回答を求めた．ただし，2問（質問4と8）では，7項目の選択肢を提示し被験者に上位3つの理由を選択させ，1位を3点，2位を2点，3位を1点と換算し集計を行った．開封の際には，封筒の消印などから被験者の特定が可能となる恐れがあるため，開封は研究者以外の第三者が行い質問票のみを集計者が受け取りデータを解析した．また，質問票の最後の設問では自由記載で被験者の意見を求めた．なお，この調査は「臨床研究に関する疫学指針」を遵守し，研究実施機関の医学研究科長と学内倫理委員会の承認を得たうえで行った．

【データ解析方法】

自由記載欄を除く各質問項目に対し，明らかに虚偽

註）CRCの呼称について：平成9年度「新GCP普及定着総合研究」により治験実施のための専従スタッフとして「治験コーディネーター」という呼称で発足．平成18年度「新たな治験活性化5カ年計画」では臨床研究の領域で広く活躍できるように「臨床研究コーディネーター」という呼称の変更を促進しており，当センターでもこの呼称を採用している．

Key words：clinical trial, questionnaire, clinical research coordinator (CRC), informed consent (IC)

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(投稿受付2009年6月18日，第2稿受付2009年7月16日，第3稿受付2009年7月28日，掲載決定2009年7月30日)

Table 本研究で使用した質問票の回答部分（前文は紙面の都合で省略）

以下の質問に対し，該当する回答に○を１つつけて答えてください．

1．今回この試験に参加されるまでに，臨床試験という言葉は知っていましたか？
①知っていた　②知らなかった
2．臨床研究コーディネーター（CRC）の存在は知っていましたか？
①知っていた　②知らなかった
3．臨床研究コーディネーター(CRC)の試験説明の補助は理解しやすかったですか？
①とても理解しやすかった
②理解しやすかった
③どちらでもない
④理解しにくかった
⑤まったく理解できなかった
☆④，⑤に○を付けたかたにお聞きします
どのような項目が分かりにくかったですか？
①手術・入院に関すること
②副作用などに関すること
③検査などの試験のスケジュールに関すること
④その他（　　）
⑤全体に分かりにくかった
4．説明を受けた後，臨床試験に参加しようと決めた理由はどれですか？
上位３つをお答えください．
1（　　）　2（　　）　3（　　）
①医師に勧められたため
②試験薬による治療効果を期待したため
③早期に治療を受けることができると思ったため
④治療費の負担が軽くなると思ったため
⑤医学・医療の発展に貢献したいと思ったため
⑥検査や入院期間中，臨床研究コーディネーター（CRC）の対応があるため
⑦その他（　　）
5．入院中の医師や臨床研究コーディネーター（CRC）の病室訪問などに満足しましたか？
①とても満足した
②満足した
③どちらでもない
④あまり満足しなかった
⑤不満足であった
☆③，④，⑤に○を付けたかたにお聞きします
どのような部分にご満足頂けなかったでしょうか？　よろしければ理由をお聞かせください．
(自由記載)
6．臨床研究コーディネーター（CRC）の検査同行には満足しましたか？
①とても満足した
②満足した
③どちらでもない
④あまり満足しなかった
⑤不満足であった
☆③，④，⑤に○を付けたかたにお聞きします
どのような部分にご満足頂けなかったでしょうか？　よろしければ理由をお聞かせください．
(自由記載)
7．臨床試験に臨床研究コーディネーター（CRC）は必要だと思いますか？
①とても必要だ
②必要だ
③どちらでもよい
④あまり必要でない
⑤必要でない
☆③，④，⑤に○を付けたかたにお聞きします
なぜどちらでもない，必要ないと思われますか？　よろしければ理由をお聞かせください．
(自由記載)
8．臨床試験を受ける上で臨床研究コーディネーター（CRC）に求めることはなんですか？　上位３つをお答えください．
1（　　）　2（　　）　3（　　）
①医療・保険について豊富な知識がある．
②試験薬について詳しく知っている．
③先生や看護師さんのパイプ役になってくれる．
④検査などに同行してくれる．
⑤試験の内容や入院について気軽に聞くことができる．
⑥試験の内容，それ以外に不安に思うことを話せる．
⑦その他
☆⑦その他　を選んだ方はよろしければその内容をお聞かせください．
(自由記載)
9．試験薬または偽薬（プラセボ）のどちらに割りつけられたかを知りたいと思いますか？
①知りたい　②知りたくない
10．この試験に参加してみてよかったと思いましたか？
①とてもそう思う
②そう思う
③どちらでもない
④そう思わない
⑤まったくそう思わない
☆③，④，⑤に○を付けたかたはよろしければ理由をお聞かせください．
(自由記載)

今回のアンケート，今回の臨床試験に関連して，ご意見・ご感想をご自由に記載してください．
(自由記載)

回答おつかれさまでした，以上で質問は終了させていただきます．
この回答用紙を返信用封筒に入れて投函してください．なお，返信用封筒には住所氏名等を無記名のまま投函していただけるようお願い申し上げます．

の回答や無回答項目が３つ以上ある場合は無効回答として処理した．なお，回答方法に誤りがあり，修正可能なものについては適切な修正を行った．以上のようにして有効回答を選出した．結果は Microsoft®Excel を用いて単純集計を行った．

4．結　果

質問票回収率は 91%（29 名/32 名）であった．また，回収された質問票のうち２名分が裏面質問に無回答であったため無効回答と判断した．したがって，回収された質問票中の有効回答率は 93%であった．以下に質問順に結果を述べる．

（自主）臨床試験についての事前の理解は約半数であり比較的認知度は高いことが分かった（質問 1）が，CRC の存在を試験参加の事前に知っていた患者はわずか 4%と少なかった（質問 2）．CRC による試験説明の補助については好意的な評価がほとんどで，「とても理解しやすかった」と「理解しやすかった」を合計すると 93%であった（質問 3）．説明が「理解しにくかった」項目は「副作用などに関すること」であった．

臨床試験に参加した理由について（質問 4，Fig. 1）は，「早期に治療を受けることができると思ったため」「医師に勧められたため」「医学・医療の発展に貢献したいと思ったため」「試験薬による治療効果を期待したため」「治療費の負担が軽くなると思ったため」「検査や入院期間中，臨床研究コーディネーター（CRC）の対応があるため」という順で回答が多かった．入院中の医師や CRC の病室訪問について（質問 5）は「とても満足した」63.0%，「満足した」37.0%と全員が好意的な評価を与えており，CRC の検査同行に対して

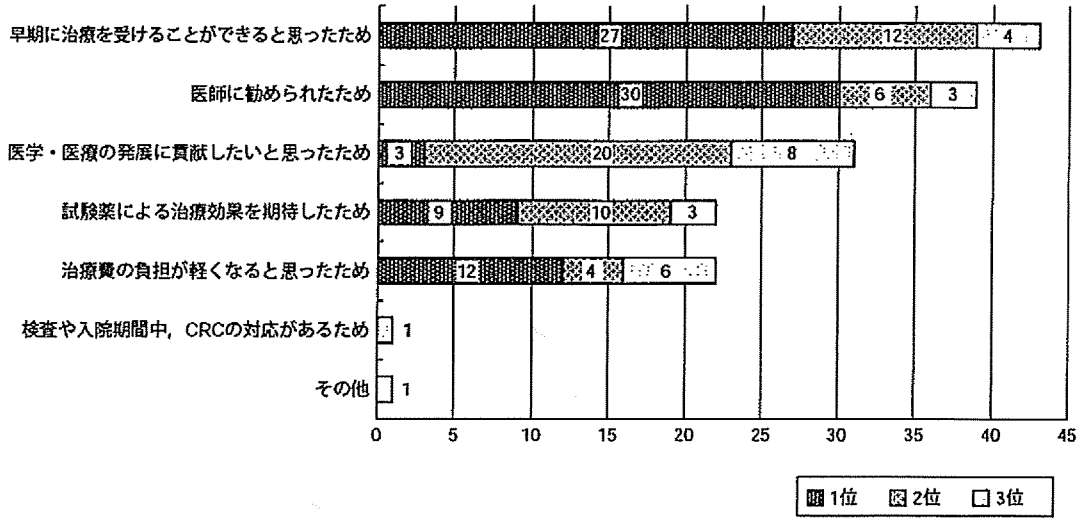

Fig. 1　臨床試験の説明を受けた後参加を決断した上位3つの理由（質問4）についての集計結果
棒グラフで表示された総合点は，1，2および3位の選択をそれぞれ3，2，1点として総合点を計算した．

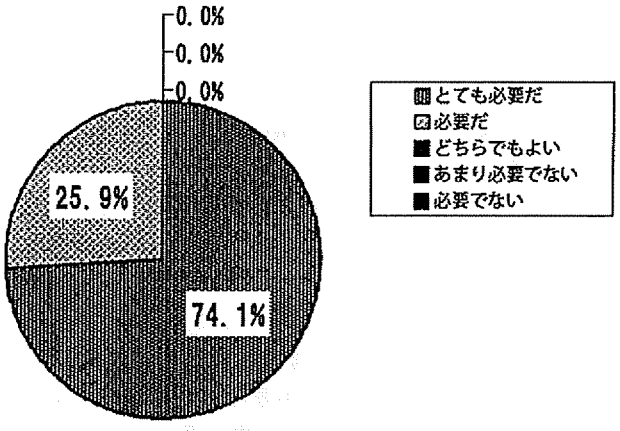

Fig. 2　臨床試験における臨床研究コーディネーター(CRC)の必要性に関する回答(質問7)の集計結果

も「とても満足した」59.3%，「満足した」40.7%と全員が満足だと回答した（質問6）．臨床試験におけるCRCの必要性について（質問7）は，「とても必要だ」74.1%，「必要だ」25.9%と全員が必要性を認識していた（Fig. 2）．臨床試験を受けるうえでCRCに求めるものについて（質問8）は，「試験の内容や入院について気軽に聞くことができる」「先生や看護師さんのパイプ役になってくれる」「医療・保険について豊富な知識がある」「検査などに同行してくれる」「試験の内容，それ以外に不安に思うことを話せる」「試験薬について詳しく知っている」の順で回答が多かった（Fig. 3）．

割り付け内容の開示を期待する被験者は67%，期待しない患者は30%であった（質問9）．試験参加への満足度については，「とてもそう思う」の回答が41%，「そう思う」が59%であり全員の被験者が好意的な評価であった（質問10）．

質問票の自由記載欄の記述を原文のまま引用すると，「試験終了後，体調が悪くなったり，なにかあると臨床試験に参加したせいではないかと不安になることがある」，「臨床試験というとモルモットというイメージがあり最初は抵抗がありました」，「実家の母から，病院の実験台になってもしものことがあったら……と最後まで反対された」，「他の患者さんから試験について聞かれたときに返答に困った」，「CRCが居ることで，入院などの費用のことや保険の手続きに関することなど，病棟スタッフに聞きづらいことが聞けてよかった」，「試験に参加したことで，日本の医療の役に立てたことも良かった」，「CRCや試験担当医師から，試験のスケジュールや次の日の検査のことを詳しく教えてもらえたのが良かった」，「毎日様子を聞きに来てもらったので，なにか話したいことがある時にすぐに話すことができ安心できた」，といった通常の質問票では得ることが困難な患者の内的体験を知ることができた．

5．考　察

本研究では自主臨床試験に参加した被験者に対する質問票調査から，臨床試験に参加した被験者のCRCに対する評価を質問票形式と自由記載によるナラティブな視点で評価することができた．結果から臨床試験を支援するCRCの周知度は低い（4%）ことが確認された．そのため，試験参加の動機として「検査や入院期間中にCRCの対応があること」を選択したのは患

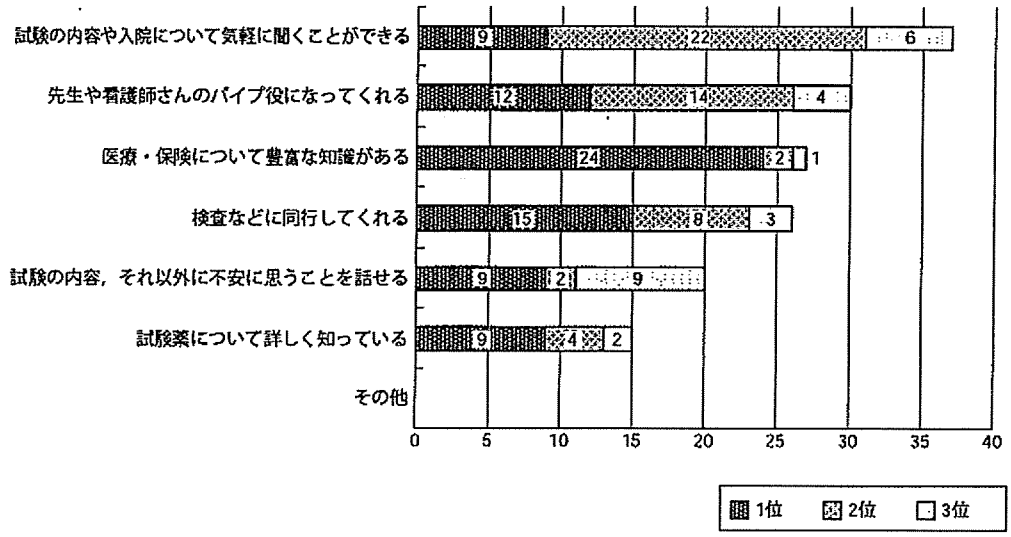

Fig. 3　臨床試験を受ける上で臨床研究コーディネーター（CRC）に求める項目（質問8）に関する集計結果
棒グラフで表示された総合点は，1，2および3位の選択をそれぞれ3，2，1点として総合点を計算した．

者1名でかつ選択順位は第3位であった．しかし，臨床試験に参加した患者の事後調査では，CRCの業務内容は高い評価を得ていた．この理由は，質問票の個別項目の回答を総合して勘案すると，今回の臨床試験では医師の説明の後に40〜60分と十分な時間をかけてCRCが患者と面談したため，被験者は事前の医師による説明では理解できなかった事項や医師には率直に聞きにくかった金銭的負担の問題や医療保険上の疑問，さらに臨床試験に関する精神的不安などについても納得できるコミュニケーションが得られたためと考えられる．また，CRCが検査に同行したり，入院中に病室を毎日訪問し面談を行うことで被験者の要望に応えたり不安を解消したことにも好印象を持っていた．以上の結果から，CRCの有用性の高さは試験参加への満足度にもつながっていることが考えられた．今後はCRCの認知度をより高め，被験者に対してどのような支援ができるのかを院内だけでなく地域，社会全体に広めていく必要があると考える．

今回の調査では，臨床試験参加の動機として「早期に治療を受けることができる」ことを27名中9名の患者が第1位に選択していた（Fig. 1）．これは，本試験の治療対象疾患が整形外科疾患で手術的介入が必要であったことが関係していると推測した．試験参加者に対しては専用の手術予定枠があらかじめ確保されていたため，試験の参加者は他の患者よりも手術待機期間が短いという利点があった．

患者全員から試験への参加についての満足度について「よかった」以上の評価が得られた．この理由は，当該試験が実施された診療科の該当疾患の手術適応者が多く，手術の待機時間が長かったため試験への参加が早期治療のインセンティブとなったことと，試験に使用された薬物の予測可能な副作用発現頻度が低かったことが関係しているものと解釈された．

本調査では，ランダム化試験に参加した患者に対して治療内容の割り付け内容の開示の有無についても回答を得たが，割り付け内容の開示を希望しない参加者は30%と想定していたよりも多かった．ただし，この結果は当該臨床試験が高齢者を対象に含めた試験であったためとも考えられ，若年者のような別の集団を対象にした場合では異なる可能性も考えられる．今後，異なる集団での検討が期待される．なお，当該臨床試験の実施計画では，割り付け内容の開示を行う予定であったが，本調査票研究の結果を受けてキーオープン後に結果のお知らせの要・不要を再度記名ハガキにて確認し，希望者に割り付け結果を送付した．

自由記載アンケートには被験者の現在の日常生活の様子，今後の大学病院での臨床試験の発展を望む声，医師・看護師・CRCへの感謝の気持ちを述べる記載があった．初めて外来で医師から試験への参加を勧められた際の不安な気持ち，その後の家族の反対，副作用に対する不安などが率直に記されていた．これらは従来臨床試験で調査されることの少ない，いわば患者のナラティブな情報であり，患者を擁護する立場にあるCRCに対しては貴重な内容であった．とくに，被験者の臨床試験参加により生じる被験者の家族に不安や悩みを知ることは重要であると考えた．今後は，医

学・医療の発展に貢献したいという被験者の思いに応えるべく，CRCは試験終了後の被験者フォローアップ，コミュニケーション力の向上などを充実させるだけでなく臨床試験の正しい理解や被験者の家族の不安を軽減するために臨床試験自体の社会的啓蒙活動も実施して行くべきと考えた．

本研究は従来知られることの少なかった臨床試験に参加した被験者のナラティブな情報に基づく試験評価を知るうえで意義が深かった．ただし，今回の調査は単一の整形外科領域の自主臨床試験に参加した比較的少数の被験者を対象にした調査であったので，この結果を一般化するには更なる研究が必要であると考える．

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FORUM

Survey of Participants' Satisfaction with an Investigator-initiated Clinical Trial and Their Appraisal of Clinical Research Coordinators

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Background：While many previous studies have examined the satisfaction of participants in clinical trials sponsored by pharmaceutical companies for the development of new drugs, few studies of subject satisfaction have been conducted on investigator-initiated clinical trials. The present study surveyed the participants' satisfaction with an investigator-initiated clinical trial and their appraisal of clinical research coordinators (CRCs).

Methods：A questionnaire was sent by post to 32 middle-aged patients who participated in a phase Ⅱ trial related to an orthopaedic disease. The questionnaire consisted of a total of 16 items. The participants were asked to fill out the questionnaires anonymously and return them by post.

Results：The response rate of the questionnaire was 91%. The contribution of CRCs to the trial was highly rated and they were considered indispensable to the clinical trial. Particularly, CRC's knowledge about the healthcare and insurance systems was given a high score, and their assistance at medical examinations during the trial was also highly rated.

Conclusion：The present study revealed the needs and potential contribution of CRCs in an investigator-initiated clinical trial for an orthopedic disease. Further studies are required to determine whether the results obtained from the present study can be generalized to clinical trials in other clinical areas.

Key words：clinical trial, questionnaire, clinical research coordinator (CRC), informed consent (IC)

〈抄録〉第29回 日本臨床薬理学会年会 2008年12月4~6日 東京
シンポジウム6：CRC研修のあり方：導入研修からadvanced研修まで

2．CRCの専門性アップに必要な研修とトレーニング：臨床試験データマネジメント

新 美 三由紀*

1．はじめに

わが国にCRC（Clinical Research Coordinator）が誕生して10年になる．多くの研修会が開催され，認定CRCも増えた．欧米と同様に，1~2年でCRCを辞めていく人も多い一方で，CRCの専門性をさらに高めたり，モニターやデータマネージャ（Data Manager：DM）等の臨床試験専門職（Clinical Research Professional：CRP）としての可能性を広げキャリアアップする人も増えている．

今回，CRCのadvanced研修を考えるにあたり，CRC業務の1つである臨床試験データマネジメント（Clinical Data Management：CDM）に焦点を当て，医療機関側のCDMにおける専門性を高める場合に必要な技術，知識，経験と，CRCキャリアパスの分化としてのDMのキャリアアップについて述べる．

2．CRCのadvanced研修を考えるにあたって

近年，職業的前進の明示的な道筋をつける装置として，キャリアラダー戦略を基本としたキャリアラダー・プログラムが全米各地で立ち上がっている．Fig.1のように，ネオリベラリズム的市場社会化が進行して貧困と格差が広がり，職業構造がピラミッド型からガビョウ型に変化したが，中間レベルの職が少なく上昇移動が困難になった．中間部分の職とそこに至る職である中間スキル層を再創出する，上昇移動が可能なキャリアのハシゴ（ラダー）をかけようという試みが，「キャリアラダー戦略」である[1]．

筒井ら[1]は，キャリアラダー戦略は看護・介護職等，「中間スキル層の厚みがあることに合理性がある業種・職種においてこそ，とる意味がある」と述べており，Fitzgerald[1]も最も適用しやすい職種として医療職を挙げている．CRCは国家免許ではないが獲得スキルの証明として「認定」が比較的有用であり，世界共通である．キャリアラダー戦略を適用しやすい職種と言えるだろう．とくにわが国では，CRCは雇用者や国家・公的組織が，訓練の機会を積極的に創り，職場の構造化を図ろうとしている職種である．CRCのステップアップにキャリアラダー戦略を取り込むことは有用と思われる．

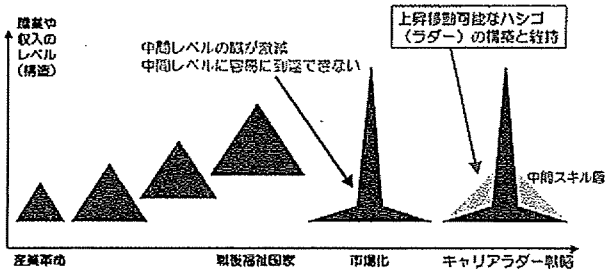

Fig. 1 職業・収入階層の構造変化とキャリアラダー戦略についての概念図[1]

中野らによって提案されたCRCの「ABC Steps」を取り入れ，米国のボストン小児病院のキャリアパスを参考に，日本のさまざまな領域の医療機関で一部以上を実現できそうなCRCのキャリアパスの例（Fig.2）を作成してみた．CRCの業務は，コーディネーション，患者対応，CDM，文書管理等，多種多様であり，臨床試験に関わる他職種と異なる点である．このため，仕事の中にいくつかのラダーを創ることで，バックグラウンドの異なる個人の特性を活かすことができると考える．

3．データマネージャとしてのキャリアアップ

CDMとは，臨床研究においてデータエラーの発生を低くコントロールし，研究を科学的・倫理的・効率的に行って正しい結論を導くための技術体系で，実践科学である[2]．CDMは，依頼者・データセンターで行われるもののみを指すのではなく，医療機関側の業務

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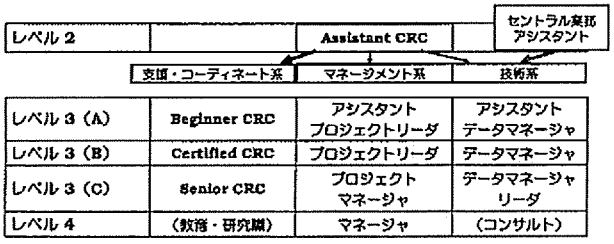

Fig. 2 CRC のキャリアパスの一例

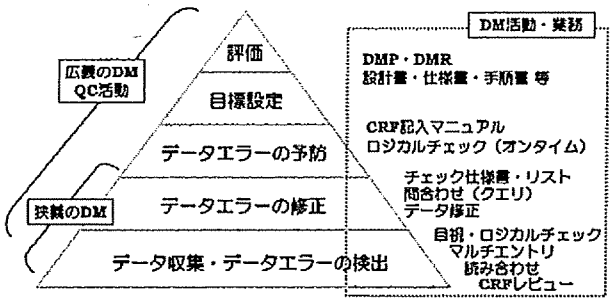

Fig. 3 データマネージメントレベルと活動

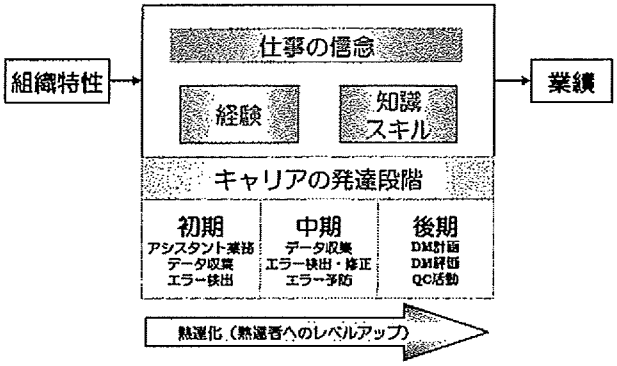

Fig. 4 データマネージャにおけるキャリアアップ

も含めた，セントラルとローカルの融合した広義の CDM（Fig. 3）が品質管理活動として重要である[3,4]．CRC のキャリアパスの一部として DM を考えるのであれば，CDM 全体を考慮し，Fig. 4 のようなキャリアの発達段階を設定する必要がある[5]．

DM としての熟達化を実現するには，知識とスキルと経験が必要である．知識を得るには，やはり CDM の基礎から習得できるセミナーが最適であり，CDM の基礎は依頼者側であっても医療機関側であっても共通している．また，CDM が実践科学である以上，フィールドでの経験は極めて重要であり，その領域の熟達者[5]になるにはフィールドを離れないことが求められるだろう．

4．まとめ

CRC は多種多様な業務に対応する専門職であるため，advanced 研修を考える際，その職種の特殊性を考慮するとキャリアパスは 1 つではない．多様性を認めるフレキシビリティが求められる．CRC 個人も，キャリアアップを実現するには，各領域の知識や技術を身につけるだけでなく，個人の能力を活かすキャリアパスにおいて経験を積むことが良いだろう．

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ORIGINAL ARTICLE

Phase II study of S-1 and docetaxel for previously treated patients with locally advanced or metastatic non-small cell lung cancer

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Abstract
Purpose The purpose of the present phase II study was to evaluate both the efficacy and toxicity of the combination of S-1 and docetaxel in previously treated patients with locally advanced or metastatic non-small cell lung cancer.
Methods Thirty-eight previously treated patients with non-small cell lung cancer were treated with S-1 (80 mg/m^2, days 1–14, oral) and docetaxel (40 mg/m^2, day 1, intravenous) every 3 weeks.
Results No complete response was observed, and seven patients had a partial response, yielding an overall response rate of 18.4% (95% CI, 7.7–34.3%). The median overall survival time and 1-year overall survival rate were 16.1 months and 60%, respectively. The median progression-free survival time was 4.4 months. Myelosuppression was the main toxicity with grade 3 or 4 neutropenia and leukopenia in 50 and 21%, respectively. There was no irreversible toxicity in this study.
Conclusions The combination of S-1 and docetaxel is well tolerable and has substantial activity for patients with locally advanced or metastatic non-small cell lung cancer. A phase III trial comparing docetaxel with or without S-1 would warrant further investigation.

Keywords Non-small cell lung cancer · Phase II study · Docetaxel · S-1 · Second-line chemotherapy · Third-line chemotherapy

Kazuhiro Yanagihara, Kenichi Yoshimura and Miyuki Niimi equally contributed to this work.

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Introduction

Non-small cell lung cancer (NSCLC) is a leading cause of cancer deaths worldwide, but only a minority of patients is amenable to surgical or definitive chemoradiotherapy. The overall prognosis of NSCLC patients remains poor; only 15.2% patients are alive after 5 years [1]. Almost all patients eventually experience progression during or after treatment. Second-line chemotherapy with docetaxel showed modest antitumor activity, with overall response rate (ORR) of 6.7–7.1%, and can prolong survival after failure of platinum-based regimens for NSCLC, with a 1-year overall survival (OS) rate of 21–31% [2, 3]. However, despite current evidences supporting the use of second-line chemotherapy, the modest survival benefits, the negligible low response rate and relevant toxicity may reduce the role of second-line chemotherapy in clinical settings.

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S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) is an oral fluoropyrimidine agent comprising the 5-fluorouracil (5-FU) prodrug tegafur and two enzyme inhibitors, 5-chloro-2,4-dihydroxypyrimidine (CDHP) and potassium oxonate (OXO), in a molar ratio of 1:0.4:1. CDHP enhances the serum 5-FU concentration by competitive inhibition of dihydropyrimidine dehydrogenase (DPD), an enzyme responsible for 5-FU catabolism. OXO is a reversible competitive inhibitor of orotate phosphoribosyl transferase (OPRT), a phosphoenzyme for 5-FU and reduces the gastrointestinal toxicity of 5-FU [4]. These mechanisms mean that oral S-1 administration can generate a higher concentration of 5-FU than protracted intravenous injection of 5-FU alone, while the incidence of toxicity in the gastrointestinal tract does not increase.

The combination of S-1 and docetaxel holds particularly great promise because both drugs have substantial antitumor activity as single agents, and they have different mechanisms of action and different toxicity profiles [2, 3, 5–7]. Recent preclinical studies have shown that S-1 has synergistic effects in human cancer xenografts [8–10]. The low level of DPD, thymidylate synthase activities, and a high level of OPRT activity enhance the antitumor effect of 5-FU and S-1. Docetaxel is one of the agents that modulate these enzyme expressions and activities. A phase I/II study has shown that this combination was well tolerated with moderate toxicities and promising activity in patients with gastric cancer [11]. Therefore, we conducted a phase II study to evaluate both the efficacy and toxicity of S-1 combined with docetaxel in previously treated patients with locally advanced or metastatic NSCLC.

Materials and methods

Eligibility criteria

Eligible patients were aged 20–74 years and had histologically or cytologically confirmed locally advanced or metastatic NSCLC (stages IIIB–IV or relapse after surgery) that progressed after first- or second-line chemotherapy or chemoradiotherapy. The patients were required to have measurable disease by the response evaluation criteria in solid tumors (RECIST), an Eastern cooperative oncology group (ECOG) performance status (PS) of 0 or 1, ability to take oral medication and normal ECG. Eligibility requirements also included a white blood cell count of $\leq$12,000 cells/mL, an absolute neutrophil count of $\geq$2,000 cells/mL, a platelet count of $\geq$100,000 cells/mL, a hemoglobin level of $\geq$9 g/dL, a serum total bilirubin level of $\leq$1.5 mg/dL, a serum aspartate aminotransferase (AST)/alanine aminotransferase (ALT) of less than or equal to twice the upper limit of normal, a serum creatinine level of $\leq$1.5 mg/dL and a normal electrocardiogram. Prior thoracic radiotherapy was allowed as long as it had been completed at least 12 weeks prior to inclusion and the patient had recovered from any toxicity. At least 4 weeks had to have elapsed from prior surgery and completion of prior chemotherapy or chemoradiotherapy. Patients who had exhibited evidence of severe heart or pulmonary disease or concomitant malignancy were excluded. The protocol was approved by the Ethics Committee of Kyoto University, and every patient gave written informed consent. This trial was registered at University hospital Medical Information Network, Japan (protocol ID number, UMIN000000501 at http://www. umin.ac.jp/).

Treatment plan

S-1 was given orally twice daily for 2 weeks, followed by a drug-free interval of 1 week (one cycle). Dose of S-1 administered each time was calculated according to the patient's body surface area as follows: less than 1.25 m^2, 40 mg; 1.25–1.5 m^2, 50 mg; and greater than 1.5 m^2, 60 mg. Docetaxel intravenous infusion (40 mg/m^2) was administered on day 1. The treatment regimen was repeated every 3 weeks until disease progression or intolerable toxicity occurred. For patients who experienced febrile neutropenia, hemorrhage with grade 3 or 4 thrombocytopenia, or grade 3 or 4 non-hematologic toxicity, the dose of docetaxel was to be reduced to 35 mg/m^2 and the dose of S-1 was also to be reduced to 80% of the initial dose. For patients who still experienced the same toxicity after the dose reduction, S-1 was to be reduced to 80% of the reduced dose, and this could be done up to twice. If recovery from such toxicities at a reduced dose was confirmed, administration at the reduced dose was continued. Patients who still experienced the same toxicity after the dose reduction were to be withdrawn from the study treatment.

Evaluation of response and toxicity

Patients underwent tumor assessments at baseline and every 6 weeks by investigators using RECIST. Patient survival was observed until death, loss to follow up, or study closure. Adverse events were recorded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3).

Statistical analysis

The primary end point was the ORR as assessed in all eligible and treated patients, with success being defined as a complete response (CR) or partial response (PR) according to RECIST. The secondary endpoints were OS, progression-free survival (PFS) and adverse events. The design of

this study was based on a binomial distribution with no planned interim analysis. Assuming a null hypothesis of a 9% ORR and an alternative hypothesis of a 25% ORR, with one-sided type I error = 0.1 and type II error = 0.1, it was necessary to enroll a minimum of 35 patients. According to this, we aimed for 40 patients to take non-evaluable patients into consideration.

Exact confidence interval (CI) and exact *P*-value for ORR were based on the binomial distribution. OS was calculated from the date of registration until death from any cause, whereas PFS until disease progression or death from any cause. OS and PFS were analyzed using the Kaplan–Meier method. All statistical tests were one sided, and a *P*-value of less than 0.05 was considered statistically significant. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Between August 2006 and December 2007, 42 patients were enrolled in this study according to the eligibility criteria. Thirty-nine of these 42 patients were eligible, of the remainder one patient had stage IIIA NSCLC and two patients were without adequate liver function. Following the study protocol, one eligible but untreated patient was excluded from the analysis because of the incidence of a compression fracture caused by osteoporosis before treatment. Baseline characteristics of the 38 patients are summarized in Table 1. The median age was 65 years (range, 44–74 years). The majority of patients had an ECOG PS of 0 (95%), had been histologically or cytologically diagnosed as having adenocarcinoma (79%) and had progressed after at least one previous platinum-based chemotherapy regimen (92%). The median number of courses administered per patient was five (range, 1–8). The median follow-up time was 17.2 months.

Efficacy

Tumor response results are shown in Table 2. Among all treated patients, no CR was observed and seven patients had a PR, yielding an ORR of 18.4% (95% CI, 7.7–34.3%; $P = 0.05$ under the null hypothesis of a 9% ORR). Among the patients with adenocarcinoma, PR was observed in 4/30 (13.3%). As shown in Fig. 1, the median OS time was 16.1 months and the 1-year OS rate was 60% (95% CI, 42.5–73.6%). The 1-year OS rates in stage-IIIB patients, stage-IV patients and patients with relapse after surgery were 70, 42 and 80%, respectively. The median PFS time was 4.4 months, and the 1-year PFS rate was 37% (Fig. 1).

Table 1 Patient characteristics ($n = 38$)

	No. of patients (%)
Median age	65 years; range, 44–74 years
Gender	
Male	23 (61%)
Female	15 (40%)
Histology	
Adenocarcinoma	30 (79%)
Squamous cell carcinoma	4 (11%)
Others	4 (11%)
Stage	
IIIB	10 (26%)
IV	18 (47%)
Relapse after surgery	10 (26%)
IIIB[a]	3 (8%)
IV[a]	7 (18%)
ECOG PS	
0	36 (95%)
1	2 (5%)
Smoking history	
Current/former	24 (63%)
Never	14 (37%)
Number of previous chemotherapy regimens	
1	23 (61%)
2	15 (39%)
Previous chemotherapy	
Platinum-containing	35 (92%)
Gefitinib	7 (18%)

ECOG PS Eastern Cooperative Oncology Group performance status

[a] Restaging after relapse

Table 2 Overall response rates according to RECIST ($n = 38$)

CR	PR	SD	PD	NE	ORR
0	7	25	6	0	18.4% (95% CI, 7.7–34.3%)

RECIST response evaluation criteria in solid tumors, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *ORR* overall response rate, *CI* confidence interval

Safety

The major adverse events are shown in Table 3. The most frequent hematological toxicity was neutropenia with grade 3 or 4 neutropenia observed in 50% of patients. Of these events, grade 4 neutropenia was observed in seven patients (18%) and febrile neutropenia in one patient (3%). Grade 3 or 4 leukopenia was reported in 21% of patients. The non-hematological grade 3 toxicities were anorexia in five

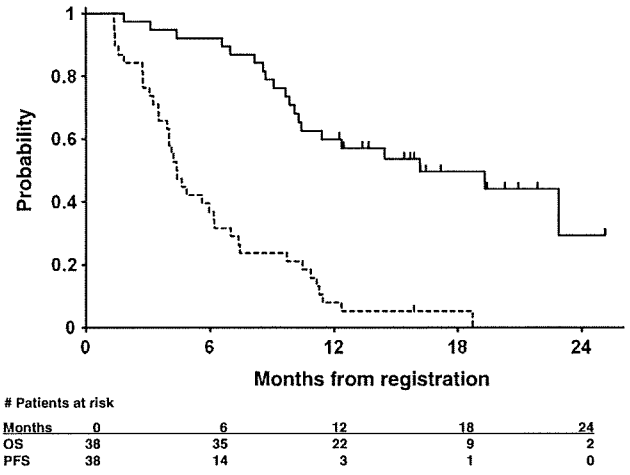

Fig. 1 Kaplan–Meier survival curves demonstrating overall (*solid line*) and progression-free (*dashed line*) survival. *OS* overall survival, *PFS* progression-free survival

Table 3 Adverse effects according to National Cancer Institute Common Terminology Criteria for Adverse Events (version 3) ($n = 38$)

Toxicity	All grades		Grade 3 or 4	
	No.	(%)	No.	(%)
Hematological toxicity				
Neutropenia	31	(82%)	19	(50%)
Leukopenia	22	(58%)	8	(21%)
Anemia	18	(47%)	1	(3%)
Thrombocytopenia	3	(8%)	0	
Febrile neutropenia[a]	1	(3%)	1	(3%)
Gastrointestinal toxicity				
Stomatitis	33	(87%)	4	(11%)
Nausea	17	(45%)	0	
Vomiting	7	(18%)	1	(3%)
Diarrhea	2	(5%)	2	(5%)
Metabolic/laboratory				
AST	15	(39%)	0	
Hyperbilirubinemia	10	(26%)	1	(3%)
ALT	10	(26%)	0	
Hypercreatinemia	3	(8%)	0	
Other toxicity				
Anorexia	25	(66%)	5	(13%)
Hand-foot skin reaction	25	(66%)	2	(5%)
Fatigue	24	(63%)	0	
Hyperpigmentation	8	(21%)	–	
Weight loss	4	(11%)	0	
Pneumonitis	2	(5%)	0	

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] Fever with concomitant grade 3 or 4 neutropenia

patients (13%), stomatitis in four patients (11%), hand-foot skin syndrome in two patients (5%), diarrhea in two patients (5%) and vomiting in one patient (3%). There was no death or irreversible toxicity in this study that was considered to be related to treatment.

Discussion

Almost all patients with advanced NSCLC treated with first-line chemotherapy experience progression, and current options for the second-line treatment of NSCLC include single-agent chemotherapy with docetaxel, pemetrexed or erlotinib [12], which large-scale randomized clinical trials indicate as the standard regimen. However, the clinical responses to these agents are of short duration, and the survival benefit is limited.

Many reports have been published investigating combination chemotherapy using two non-platinum agents for recurrent NSCLC in randomized clinical trials, with the objective of improving outcomes further. However, none of these studies have demonstrated improved survival with combination chemotherapy, whereas there have been relatively higher or intolerable toxicities [13–16]. Therefore, more active regimens for the second-line chemotherapy are much needed.

In the present study, we evaluated the efficacy and safety of the combination of S-1 and docetaxel, two agents that separately have shown promise in the treatment of advanced or metastatic NSCLC. This combination chemotherapy conferred efficacy with an ORR of 18%, a median OS time of 16 months and a 1-year OS rate of 60%. The 18% ORR observed in this study was slightly lower than expected. However, the survival benefits as second- or third-line therapy observed compare favorably with other chemotherapy regimens, such as monotherapy with docetaxel (6–14 months) [2, 3, 7], pemetrexed (8 months) [17], erlotinib (6–15 months) [18–20] or oral topotecan (6–8 months) [21, 22], or combination chemotherapy of irinotecan and cisplatin (11 months) [23], or oral fluoropyrimidine UFUR and gemcitabine (13 months) [24], although between-study comparisons should be made with caution.

Prolonged survival may be due to substantial post-study treatment, especially epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs). Although EGFR mutation status was not analyzed in this study, 17 patients received EGFR-TKIs and 9 of those patients for over a month.

The hematological toxicity observed here was minimal and tolerable, despite the fact that grade 3 or 4 neutropenia occurred in 50%, which is comparable with the toxicity caused by docetaxel monotherapy. The majority of non-hematologic toxicities were mild and tolerable without grade 4 non-hematologic toxicity. These toxicity results are consistent with those observed in a phase I/II study in patients with gastric cancer [11].

During the preparation of this manuscript, Atagi et al. [25] reported the results of a phase I/II study, in which the combination of S-1 and docetaxel was evaluated for patients who had failed one or more prior chemotherapy regimens. In the phase II part of their study, seven of 29 eligible patients achieved a PR, yielding an ORR of 24%, with a median OS time and the 1-year OS rate of 12 months and 42%, respectively. Patient characteristics were similar except for stage and ECOG PS: fewer patients who had experienced relapse after surgery were included, and 31 and 69% patients had ECOG PS of 0 and 1, respectively, in the study by Atagi et al. [25]. Although these differences in patient characteristics may lead to more favorable survival results in our study, the combination of S-1 and docetaxel still seems to be consistently promising as a chemotherapy option after the failure of prior chemotherapy for advanced NSCLC.

In this study, the dose of docetaxel was lower than that commonly used in docetaxel monotherapy. As a second-line docetaxel monotherapy, a dose of 75 mg/m^2 every 3 weeks is used in the United States and Europe, and the dose is 60 mg/m^2 every 3 weeks in Japan. However, our regimen is widely recognized as a tolerable and optimized combination of S-1 and docetaxel in gastric cancer [11], and thus, also in lung cancer, it is considered promising in terms of toxicity and efficacy. Furthermore, it was the recommended dose in the phase I part of study reported by Atagi et al. [25].

There are many report of ethnic differences in the safety and efficacy profile of S-1 and docetaxel [25–28], and it is shown that CYP2A6*9 genetic polymorphism is a potential predictive marker, for efficacy and toxicity, for the patients received the combination of S-1 and docetaxel for metastatic gastric carcinoma [29]. In the development of a S-1/docetaxel combination therapy in the United States and Europe, further optimization of the dose of each agent may be required to account for these differences.

In conclusion, the combination of S-1 and docetaxel is well tolerable and promisingly effective for patients with locally advanced or metastatic NSCLC. A phase III trial comparing docetaxel with or without S-1 would warrant further investigation.

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Associations between glutathione S-transferase π Ile^{105}Val and glyoxylate aminotransferase Pro^{11}Leu and Ile^{340}Met polymorphisms and early-onset oxaliplatin-induced neuropathy

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ABSTRACT

Purpose: Although the risk of oxaliplatin-induced neuropathy depends on cumulative oxaliplatin dose, susceptibility to this adverse event differs greatly among patients. In this study, we investigated the associations between oxaliplatin-induced neuropathy and the following polymorphisms: glutathione S-transferase π (*GSTP1*) Ile^{105}Val, and glyoxylate aminotransferase (*AGXT*) Pro^{11}Leu and *AGXT* Ile^{340}Met. *Experimental design*: Eighty-two Japanese patients with histologically confirmed colorectal cancer who received at least six cycles of the modified FOLFOX6 (m-FOLFOX6) regimen were enrolled. To minimize differences in cumulative oxaliplatin dose between patients, oxaliplatin-induced neuropathy was evaluated using an oxaliplatin-specific scale during the 2-week period after completion of the sixth cycle of treatment. *Results*: Forty-four patients developed grade 2/3 oxaliplatin-induced neuropathy. There were more patients carrying at least one *GSTP1* ^{105}Val allele among the group with grade 2/3 neuropathy (18/44, 41%) than among the group with grade 1 neuropathy (9/38, 24%), although the difference was not statistically significant ($P = 0.098$). There were similar numbers of patients carrying at least one *AGXT* ^{105}Met allele in the grade 2/3 neuropathy (7/44, 16%) and grade 1 neuropathy groups (5/38, 13%; $P = 0.725$). The *AGXT* ^{11}Leu allele was not found in any of our patients or controls. *Conclusions*: We found no significant association between oxaliplatin-induced neuropathy and the *GSTP1* Ile^{105}Val and *AGXT* Ile^{340}Met polymorphisms. Given that no *AGXT* ^{11}Leu allele was found among our study population ($n = 177$), evaluating this polymorphism in Japanese patients in future studies is likely to be uninformative.

1. Introduction

Oxaliplatin, a third-generation diaminocyclohexane platinum compound, is a key drug in the chemotherapeutic treatment of patients with advanced colorectal cancer [1–3]. Oxaliplatin is widely used in palliative settings, and in recent times its efficacy in neoadjuvant and adjuvant settings has also been established, thus an increasing number of colorectal cancer patients are receiving this drug [4,5]. Oxaliplatin often causes neuropathy, which limits the ongoing use of this drug in spite of its wide range of efficacy. The risk of developing oxaliplatin-induced neuropathy depends on cumulative oxaliplatin dose [2,6,7]. However, susceptibility to oxaliplatin-induced neuropathy differs greatly among patients. Some patients suffer from persistent neuropathy for more than 2 years after withdrawal of oxaliplatin, whereas others can tolerate a cumulative oxaliplatin dose of more than 800 mg/m^2 without experiencing neuropathy [6–9]. Since there is currently no effective treatment for oxaliplatin-induced neuropathy, risk assessments for this adverse event using a pharmacogenetic approach are clinically valuable.

To date, several genes have been identified as being of interest in the context of the efficacy and toxicity of oxaliplatin.

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Glutathione S-transferase π (*GSTP1*) is a xenobiotic-metabolizing enzyme involved in the detoxification of a variety of chemotherapeutic drugs, including platinum derivatives [10]. Rs1695, a non-synonymous single nucleotide polymorphism (SNP) of *GSTP1*, converts Ile to Val at codon 105 and reportedly alters the enzymatic activity of the molecule [10–13]. Given that altered *GSTP1* enzyme activity is likely to affect the detoxification of platinum drugs, the association between the *GSTP1* Ile^{105}Val polymorphism and clinical response to platinum-based chemotherapy has been examined [14–28]. However, few of these studies were designed to evaluate oxaliplatin-induced neuropathy as a primary endpoint. In one such study, that by Lecomte et al., it was found that grade 3 neuropathy was significantly more frequent among patients harboring the homozygous *GSTP1* ^{105}Ile allele than among patients with other genotypes. These authors hypothesized that the GSTP1 ^{105}Ile protein weakens the cell's defenses against oxaliplatin neurotoxicity via inhibition of c-Jun NH_2-terminal kinase activity [17]. The results of several other studies support this hypothesis [25,27], whereas other groups have reported no significant association between this genotype and oxaliplatin neurotoxicity [24,26] or have obtained contradictory results [21,28]. Other experimental findings that indirectly challenge the view of Lecomte et al. are that the GSTP1 ^{105}Val protein is a less potent detoxifier of carcinogens than the GSTP 1 ^{105}Ile protein [13] and that patients with the Val/Val genotype have been found to receive significant survival benefit from oxaliplatin [14]. Thus, the role of the *GSTP1* Ile^{105}Val polymorphism in predicting oxaliplatin-induced neuropathy is still controversial.

Oxaliplatin affects neural voltage-gated sodium channels indirectly via one of its metabolites, oxalate [29,30]. Based on the fact that glyoxylate aminotransferase (AGXT) is involved in the oxalate metabolic pathway [31], Gamelin et al. hypothesized that alterations in *AGXT* genotype and therefore enzyme activity due to *AGXT* genotype could theoretically affect the likelihood of oxaliplatin-induced neuropathy. In fact, they found that the *AGXT* Pro^{11}Leu and Ile^{340}Met minor polymorphisms are significantly associated with a higher risk of oxaliplatin-induced neuropathy [24].

However, all the above-mentioned studies were carried out using Caucasian patient populations, and to date there have been no such studies performed for Asian patients. There exist considerable interethnic differences in genotype frequency, which can greatly affect the results of pharmacogenetic analyses. For example, the *UGT1A1*28* marker is known to vary markedly between ethnic groups [32–34].

In the present study, we investigated the association between the *GSTP1* Ile^{105}Val, *AGXT* Pro^{11}Leu and *AGXT* Ile^{340}Met polymorphisms and the development of oxaliplatin-induced neuropathy in Japanese colorectal cancer patients. To minimize differences in cumulative oxaliplatin dose, we studied oxaliplatin-induced neuropathy that developed in the 2 weeks after completion of the sixth cycle of m-FOLFOX6.

2. Patients and methods

2.1. Patients

Between October 2005 and December 2008, a total of 174 patients with histologically confirmed colorectal cancer received at least six cycles of the m-FOLFOX6 regimen at two medical centers. Eighty-two patients (the patients' characteristics are summarized in Table 1) were enrolled in this cohort study. The remaining patients were excluded because they died ($n = 60$), were referred to another hospital ($n = 10$), or were lost to follow-up ($n = 22$) before we could obtain informed consent or a blood sample. The local ethics committees of both centers approved the study protocol. All patients enrolled in this study provided written informed consent. Patient registration and data management were conducted at a data center at Kyoto University Hospital (Translational Research Center). Forty-eight of the 82 patients were chemonaïve and the others had previously undergone at least one chemotherapy regimen other than oxaliplatin-based chemotherapy before m-FOLFOX6 (see Table 1 for details). None of the patients had a history of diabetic neuropathy, but two patients had a history of spondylosis. The m-FOLFOX6 regimen consists of 85 mg/m^2 oxaliplatin plus a 400 mg/m^2 bolus of 5-fluorouracil and 200 mg/m^2 L-leucovorin on day 1, and thereafter a 46-h infusion of 2400 mg/m^2 5-fluorouracil every 2 weeks [35]. The dose and schedule for m-FOLFOX6 were adjusted at the discretion of individual physicians according to baseline bone marrow function or the occurrence of adverse events during the previous cycle. Bevacizumab was concomitantly administered to two patients. Blood tests and physical examinations, including evaluation of oxaliplatin-induced neuropathy, were performed before each cycle. Instances of oxaliplatin-induced neuropathy that occurred during the 2 weeks after completion of the sixth cycle of m-FOLFOX6 were graded using an oxaliplatin-specific scale (grade 1: paresthesia, dysesthesia of short duration; grade 2: paresthesia, dysesthesia persisting between cycles; grade 3: paresthesia, dysesthesia causing functional impairment) [6,36]. In this study, we classified patients without paresthesia or dysesthesia into the grade 1 group.

Table 1
Patient characteristics ($n = 82$).

Variable	n (%)
Gender	
Male	51 (62)
Female	31 (38)
Age (years)	
Median	64
Range	41–80
Location of primary lesion	
Colon	47 (57)
Rectum	35 (43)
Histology	
Well-differentiated	11 (13)
Moderately differentiated	58 (71)
Poorly differentiated	1 (1)
Other	12 (15)
History of prior chemotherapy	
None	48 (59)
Yes	34 (41)
Previous chemotherapy regimen[a]	
UFT/Uzel	17
5-FU/leucovorin	9
TS-1	7
FOLFIRI	5
UFT	5
Other	3
Cumulative oxaliplatin dose (mg/m^2)	
Median	510
Range	336–510

[a] Several patients received more than one regimen.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from whole blood using the phenol–chloroform extraction method and stored at 4 °C until use. The *GSTP1* Ile^{105}Val, *AGXT* Pro^{11}Leu and *AGXT* Ile^{340}Met genotypes were determined by using a fluorescence quenching probe (QProbe, Bex Co., Ltd., Tokyo, Japan) [37,38]. Briefly, a QProbe contains cytosine at its 5′ or 3′ end, which is labeled with a fluorophore that is quenched by guanine. When a QProbe

Table 2
Genotype and allele frequencies for the investigated polymorphisms.

Polymorphism	rs ID	Nucleotide		Amino acid		Sample set	Genotype distributions (%)			Freq. A2	*P*-Value[a]
		ref. (A1)	var. (A2)	ref.	var.		A1/A1	A1/A2	A2/A2		
GSTP1 Ile[105]Val	rs 1695	A	G	Ile	Val	Grade 2/3	26 (59.1)	17 (38.6)	1 (2.3)	0.216	
						Grade 1	29 (76.3)	8 (21.0)	1 (2.6)	0.132	0.158
						Control[b]	68 (73.9)	21 (22.8)	3 (3.2)	0.147	0.155
AGXT Ile[340]Met	rs 4426527	A	G	Ile	Met	Grade 2/3	37 (84.1)	6 (13.7)	1 (2.3)	0.091	
						Grade 1	33 (86.8)	5 (13.2)	0	0.066	0.553
						Control[b]	82 (91.1)	8 (8.9)	0	0.044	0.132

Polymorphism	Sample set	Genotype distribution		*P*-Value[a]	Odds ratio[c] (95% CI)	References
		A1/A1	A1/A2 + A2/A2			
GSTP1 Ile[105]Val	Grade 2/3	26	18			[14,16]
	Grade 1	29	9	0.098	2.23 (0.86–5.82)	
	Control[b]	68	24	0.080	1.96 (0.92–4.20)	
AGXT Ile[340]Met	Grade 2/3	37	7			[23]
	Grade 1	33	5	0.725	1.25 (0.36–4.31)	
	Control[b]	82	8	0.226	1.94 (0.66–5.74)	

[a] *P*-values are calculated for grade 2/3 vs. grade 1 or grade 2/3 vs. control.
[b] A sample set representing a control population of healthy Japanese subjects was used.
[c] Odds ratios are calculated for grade 2/3 vs. grade 1 or grade 2/3 vs. control.

hybridizes with the target DNA, its fluorescence is quenched by the guanine in the target that is complementary to the modified cytosine. By monitoring fluorescence intensity, each genotype can be determined. The frequencies of the three SNPs in the general Japanese population were also examined using DNA samples from healthy Japanese volunteers in Pharma SNP Consortium (Tokyo, Japan) [39]. This population is referred to hereafter as the control Japanese population. The investigators performing the genetic analysis were blinded to the patients' characteristics and clinical condition. The characteristics and frequencies of the studied polymorphisms are shown in Table 2.

2.3. Statistical analysis

The primary endpoint of the study was the association between genotype distribution and the occurrence of grade 2/3 oxaliplatin-induced neuropathy during the 2 weeks after completion of the sixth cycle of m-FOLFOX6 treatment. The planned sample size ($n = 82$) was specified in the protocol to provide 80% power to detect an odds ratio (OR) of 5.4. The calculations were based on a previous finding that the OR of developing grade 3 oxaliplatin-induced neuropathy in a patient without the *GSTP1* ^{105}Val allele was 5.54 [17]. We estimated that the frequency of patients in our Japanese population with at least one *GSTP1* ^{105}Val allele was 21% and used estimated incidences of grade 2/3 oxaliplatin-induced neuropathy after the first six cycles of m-FOLFOX6 for patients with and without a *GSTP1* ^{105}Val allele of 30% and 70%, respectively. Statistical analysis was performed using a two-sided χ^2/Fisher's exact test with a significance level of 5%, and quantified by calculating ORs with 95% confidence intervals (95% CI). All statistical analysis was conducted using SAS (version 9.13, SAS Institute Inc., Cary, NC).

3. Results

Patient characteristics are summarized in Table 1. The median cumulative dose of oxaliplatin was 510 mg/m^2 (range, 336–510 mg/m^2). Although some patients received a lower cumulative dose than expected, the most common cause of dose adjustment was neutropenia and none of our patients underwent a dose reduction because of neurotoxicity. Forty-four patients (54%) developed grade 2/3 oxaliplatin-induced neuropathy (as scored using the oxaliplatin-specific scale) during the 2 weeks after completion of the sixth cycle of m-FOLFOX6. No significant differences in age, gender or cumulative oxaliplatin dose were observed between the group of patients with grade 2/3 neuropathy and those with grade 1 neuropathy (Table 3).

We performed genotyping analysis for the rs1695, rs34116584 and rs4426527 SNPs, which correspond, respectively, to the *GSTP1* Ile^{105}Val, *AGXT* Pro^{11}Leu and *AGXT* Ile^{340}Met polymorphisms. We performed this analysis for 44 patients with grade 2/3 neuropathy (group A) and 38 patients with grade 1 neuropathy (group B). The frequencies of the variant allele in rs1695 (G), corresponding to *GSTP1* ^{105}Val, were 0.216 for group A and 0.132 for group B. Although the frequency was higher in group A, the difference was not statistically significant ($P = 0.158$) (Table 2). The frequency of allele G in the control Japanese population was 0.147, which is similar to that of group B. When a dominant model for the variant allele (G) was applied for comparison, the number of patients carrying at least one variant allele was higher in group A (18/44, 41%) than in group B (9/38, 24%) although the difference was not statistically significant ($P = 0.098$) (Table 2). A similar trend was observed when the same comparison between group A and the control Japanese population was performed ($P = 0.080$) (Table 2).

Table 3
Association between clinical variables and genotypes and oxaliplatin-induced neuropathy.

	n (%)			*P*[a]
	Grade 1	Grade 2	Grade 3	
Age (years)				
<60	15 (62)	9 (38)	0 (0)	0.13
≥60	23 (40)	34 (59)	1 (2)	
Gender				
Male	27 (53)	24 (47)	0 (0)	0.11
Female	11 (36)	19 (61)	1 (3)	
Cummulative oxaliplatin dose (mg/m^2)				
<510	18 (49)	18 (49)	1 (3)	0.50
510	20 (44)	25 (56)	0 (0)	
GSTP1 Ile^{105}Val				
Ile/Ile	29 (52)	26 (47)	0 (0)	0.16
Ile/Val	8 (32)	16 (64)	1 (4)	
Val/Val	1 (50)	1 (50)	0 (0)	
AGXT Ile^{340}Met				
Ile/Ile	33 (48)	36 (51)	1 (1)	1.00
Ile/Met	5 (45)	6 (55)	0 (0)	
Met/Met	0 (0)	1 (100)	0 (0)	

[a] Fisher's exact test.

Table 4
Findings from published studies on *GSTP1* Ile^{105}Val and oxaliplatin-induced neuropathy among patients with colorectal cancer.

Study	Year	Sample size	Ethnicity	Regimen	Cumulative oxaliplatin dose (mg/m^2)	Genotype distributions (%)			Genotype associated with frequent neuropathy	*P*-Value
						A1/A1	A1/A2	A2/A2		
Lecomte et al. [17]	2005	64	Caucasian[a]	Mainly FOLFOX4 (72%)	≥500	39 (61)	20 (31)	5 (8)	A1/A1	0.02
Grothey et al. [28]	2005	288	Caucasian	FOLFOX4	≤600	120 (42)	130 (45)	38 (13)	A1/A2+A2/A2	0.03
Ruzzo et al. [21]	2007	166	Caucasian	FOLFOX4	N/A	92 (55)	62 (37)	12 (8)	A2/A2	<0.001
Gamelin et al. [24]	2007	122	Caucasian	FOLFOX4	255–2125	54 (44)	56 (46)	12 (10)	ns	ns
Pare et al. [25]	2008	126	Caucasian	FOLFOX4	≥510	44 (35)	49 (49)	20 (16)	A1/A1	0.08
Kweekel et al. [26]	2009	56	Caucasian	XELOX	≥500	25 (45)	25 (45)	6 (11)	ns	ns
Current study	2009	82	Japanese	m-FOLFOX6	≤510	55 (67)	25 (30)	2 (2)	A1/A2+A2/A2	0.1

N/A, not applicable; ns, not significant; XELOX regimen consists of 130 mg/m^2 oxaliplatin plus 1000 mg/m^2 capecitabine b.i.d. every 3 weeks.
[a] Except four African patients and one Asian patient.

The G allele of SNP rs4426527, corresponding to *AGXT* Ile^{340}Met, was present at frequencies that were not significantly different in group A and group B ($P = 0.553$) (Table 2). The T allele of SNP rs34116584, corresponding to *AGXT* ^{11}Leu, was absent in group A, group B and the control Japanese population ($n = 177$, data not shown). The frequencies of *GSTP1* Ile^{105}Val and *AGXT* Ile^{340}Met were in Hardy–Weinberg equilibrium.

4. Discussion

In this study, to minimize differences in cumulative oxaliplatin dose among patients, we attempted to evaluate oxaliplatin-induced neuropathy during the 2 weeks after completion of the sixth cycle of m-FOLFOX6. As a result, we evaluated early-onset neuropathy rather than the most severe grade of neuropathy that is associated with oxaliplatin.

Lecomte et al. reported that grade 3 oxaliplatin-induced neuropathy, as scored using the oxaliplatin-specific scale, was significantly more frequent among patients harboring the homozygous *GSTP1* ^{105}Ile allele [17]. Our planned sample size of 82 was based on these findings. In contrast to the findings of Lecomte et al., we found that grade 2/3 neuropathy was more common among patients harboring at least one *GSTP1* ^{105}Val allele, although the difference was not statistically significant (OR, 2.23; 95% CI, 0.86–5.82; $P = 0.098$; Table 2). There are several possible reasons for this discrepancy between the outcomes of two studies. As mentioned above, our findings reflect our focus on early-onset neuropathy, while Lecomte et al. studied the most severe grade of neuropathy experienced during oxaliplatin treatment. Our findings are in line with those of Grothey et al., who determined that patients harboring at least one *GSTP1* ^{105}Val allele were more likely to experience early-onset oxaliplatin-induced grade 2/3 neuropathy [28]. Because Grothey et al. studied neuropathy that developed before the cumulative oxaliplatin dose reached 600 mg/m^2, their approach is more similar to ours than that of Lecomte et al. Since functional impairment due to oxaliplatin neuropathy does not necessarily develop in a progressive manner but develops suddenly in some patients [40], it is possible that those who are prone to developing early-onset neuropathy have different genotypes from those who suffer from functional impairment due to oxaliplatin neuropathy.

Genetic differences related to ethnic background may also be a reason for the differences between the present results for Japanese patients and the previous results for Caucasian patients. In particular, because of the very low frequency of the *GSTP1* 105 Val/Val genotype among our patients as well as the control Japanese population, we cannot accurately assess the role of this genotype in predicting the likelihood of oxaliplatin-induced neuropathy. Previously published data on the *GSTP1* Ile^{105}Val polymorphism and oxaliplatin neurotoxicity are summarized in Table 4.

There has been only one prior study in which the associations between *AGXT* genotypes and oxaliplatin-induced neuropathy have been investigated [24]. In contrast to the findings of Gamelin et al., we did not see any significant association between oxaliplatin-induced neuropathy and the *AGXT* Ile^{340}Met polymorphism. This might also be explained by differences in genetic background. In particular, no patients harbored the *AGXT* ^{11}Leu allele in our study, whereas 30% of patients were found to harbor at least one *AGXT* ^{11}Leu allele in a Caucasian patient population [24]. In contrast to other clinical responses, such as tumor shrinkage or neutropenia, oxaliplatin-induced neuropathy is minimally affected by concomitantly administered drugs or genetic changes in the tumor cell, so genetic polymorphisms are particularly relevant in the context of susceptibility to oxaliplatin-induced neuropathy. In future pharmacogenetic studies, in order to obtain the most consistent results, we recommend that early-onset neuropathy be evaluated separately from the most severe neuropathy and inter-patient differences in cumulative oxaliplatin dose be minimized.

In summary, we found no significant associations between oxaliplatin-induced neuropathy during the 2-week period after the completion of six cycles of m-FOLFOX6 and the *GSTP1* Ile^{105}Val and *AGXT* Ile^{340}Met polymorphisms in Japanese colorectal cancer patients. Since no patients harbored the *AGXT* ^{11}Leu allele in the present study, it seems likely that evaluating this polymorphism in Japanese patients in future studies will not be informative.

Conflict of interest statement

None declared.

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Original Article

C-C Chemokine Receptor 2 Inhibitor Improves Diet-Induced Development of Insulin Resistance and Hepatic Steatosis in Mice

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Aim: Adipose tissue inflammation induced by macrophage infiltration through the MCP-1/CCR2 pathway is considered to play a pivotal role in the development of visceral obesity and insulin resistance. In the present study, therefore, we examined whether pharmacological inhibition of CCR2 is effective against the development of diet-induced metabolic disorders.
Methods: C57BL/6 mice were fed a high fat and sucrose diet with or without propagermanium (CCR2 inhibitor, 5 or 50 mg/kg BW/day) for 12 weeks from 6 weeks of age. Then we analyzed lipid and glucose metabolism and tissue inflammation in the liver and adipose tissues along with serum markers in those mice.
Results and Conclusion: Propagermanium treatment slightly decreased body weight gain and visceral fat accumulation in diet-induced obese (DIO) mice. Further, propagermanium suppressed macrophage accumulation and shifted adipose tissue macrophage polarization from the pro-inflammatory (M1) state to anti-inflammatory (M2) state in DIO mice. Expressions of TNF-α and MCP-1 mRNA in adipose tissue were reduced by propagermanium treatment, indicating that propagermanim suppressed inflammation in adipose tissue. Propagermanium treatment also ameliorated glucose tolerance, insulin sensitivity, and decreased hepatic triglyceride in DIO mice. Thus, propagermanium improved diet-induced obesity and related metabolic disorders, such as insulin resistance and hepatic steatosis by suppressing inflammation in adipose tissue. Our data indicate that inhibition of CCR2 could improve diet-induced metabolic disorders, and that propagermanium may be a beneficial drug for the treatment of metabolic syndrome.

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Key words; Obesity, Insulin resistance, Hepatic steatosis, Macrophage, Chemokine, Inflammation

Introduction

Metabolic syndrome, characterized by a clustering of visceral obesity, impaired glucose tolerance, hypertension, and dyslipidemia, is a major cause of type 2 diabetes mellitus and cardiovascular disease[1-4]. Visceral obesity and insulin resistance are thought to represent common underlying factors of the syndrome[5]; therefore, it is critical to clarify the mechanism by which obesity and insulin resistance develop and to establish a therapeutic method based on its mechanism.

Many reports have shown that visceral obesity is associated with chronic and low-grade inflammation, suggesting that inflammation may be a potential underlying mechanism by which visceral obesity leads to insulin resistance[6]. Indeed, visceral obesity and insulin resistance are strongly associated with systemic markers of inflammation, and clinically, inflammation

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