厚生労働科学研究費補助金

がん臨床研究事業

局所限局小細胞肺がんの集学的治療に関する研究

平成１７年度　総括研究報告書

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目　　次

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

「局所限局小細胞肺がんの集学的治療に関する研究」

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研究要旨
限局期小細胞肺がんの予後改善を目的に、「エトポシド＋シスプラチン(EP)療法と加速多分割胸部放射線療法(AH-TRT)の同時併用後のEP療法と塩酸イリノテカン＋シスプラチン（IP）療法の第III相比較試験」を全国38施設の研究グループで実施中である。本年度は、平成18年2月までに65例が登録され、総登録数は259症例となり、ほぼ順調に症例集積が進んでいる。平成18年2月の定期モニタリング結果では、主たる毒性は、好中球減少、食欲不振、発熱、感染など予期されたものであり、安全性についても十分許容範囲であった。

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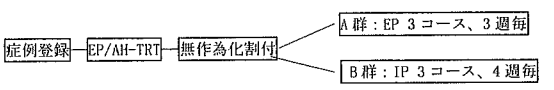
A. 研究目的
限局期小細胞肺がんの予後改善を目的として、エトポシド＋シスプラチン(EP)療法1コースと加速多分割胸部放射線療法(AH-TRT)の同時併用(EP/AH-TRT)後に、塩酸イリノテカン＋シスプラチン（IP）療法3コースを追加する治療法の有用性を検証するため、従来の標準的治療であるエトポシド+シスプラチン(EP)療法3コースの治療法を対照とした大規模第III相比較試験を計画し、適正に実施する。

B. 研究方法
［研究形式］
全国38施設の研究グループによる第III相無作為化比較試験。エンドポイントは生存期間。3年生存率を現在の30%から45%に向上させることを見込む。これは限局期小細胞肺がんの治癒率（5年生存率）を10-15%上げることに相当する。
［対象症例］
限局期小細胞肺がんの初回治療例で、70才以下、ECOG Performance Status (PS) 0-1、主要臓器機能が保持された症例。患者本人の自由意思による文書同意を必須とする。
［症例登録と無作為化割り付け］
公定書協会臨床研究データセンター（国立がんセンター研究所がん情報研究部内）での中央登録・無作為化割付け方式をとる。無作為化割り付けの調整因子は施設とPS。
［治療内容］
EP療法1コースとAH-TRTを同時併用後、EPあるいはIP療法3コースを実施する。



EP療法：　エトポシド　100 mg/m² day 1,2,3
　　シスプラチン　80 mg/m² day 1,8,15
IP療法：　イリノテカン　60 mg/m² day 1,8,15
　　シスプラチン　60 mg/m² day 1
胸部放射線療法(AH-TRT)：　45Gy/30fr./3weeks

［解析方法］

最終解析は症例集積終了5年後。中間解析2回、安全性モニタリング年2回。

［予定症例数］

270例（無作為化割り付け250例）、集積期間3年。

（倫理面の配慮）

試験実施計画書において、(1)施設 IRB の承認(2)文書を用いた十分な説明後、被験者本人の自由意思による同意(3)個人情報の厳守(4)臨床試験審査委員会、効果・安全性評価委員会による第三者的監視機構の設置を必須として、計画・実施する。

C.研究結果

1．平成15年度までの経過

平成13年に厚生労働省「21世紀型医療推進事業」に研究課題「限局期小細胞肺がんの予後改善を目指した集学的治療の研究」（主任研究者　西條長宏）を申請し、全国の肺がん診療の主要施設38施設で研究グループを組織した。実施計画書は、JCOG (Japan Clinical Oncology Group)の協力を得、平成14年7月、JCOG 臨床試験審査委員会の承認を受けた。平成14年9月より日本公定書協会臨床研究データセンターにおいて症例登録を開始した。

2．平成17年度の研究実施経過

平成17年度は、平成17年2月までに65例の登録がなされた。総登録症例数は、平成14年度（9月より）の38例、平成15年度の75例、平成16年度81例と合わせ259例となった。平成18年前半には症例登録を完了する見込みであり、当初の計画より少し遅れているものの、ほぼ順調な症例集積ペースと考える。症例集積の推進策としては、登録の少ない4施設を研究グループより削除、新たに4施設を加えた。また、研究参加施設の近隣医療機関に、本研究の紹介パンフレットを配布し、患者紹介を依頼した。

平成18年2月の定期モニタリングでは、両群の症例の主要背景因子に偏りはなく、バランスが保たれている。主たる毒性は、白血球減少、好中球減少、食欲不振、発熱、感染など予期されたものであった。グレード4の好中球減少は、導入放射線化学療法（EP/AH-TRT）、EP群、IP群の治療において、それぞれ75%、67%、29%であり、IP 療法において頻度が低かった。一方、IP 療法で最も問題とされる下痢については、グレード3の毒性を13例にみている。発熱性好中球減少は、EP/AH-TRT、EP 群、IP 群において、それぞれ27%、25%、19%に認めた。また、重篤有害事象として、肺臓炎による死亡2例（EP 群、導入EP/AH-TRT 中)、脳梗塞による死亡1例(IP群)、心筋梗塞 1 例（EP/AH-TRT 中）、高/低血糖（EP/AH-TRT 中）、低カリウム血症（IP 群）が報告されている。治癒を目指した強力な併用療法であり、安全性についても全体として、許容範囲内であると判断する。

D. 考察

我々は、進展期小細胞肺がんに対するイリノテカン+シスプラチン（IP）療法の有用性を第III相試験において検証して、新たな標準的治療法として確立させ、世界的な評価を得た。このIP療法を限局期小細胞肺がんの放射線化学療法に組み込んだ本研究は、限局期小細胞肺がんに対する新たな標準的治療の確立のための最重要課題であると同時に独創的な研究であるといえる。本研究では、症例数および質の確保の点から全国の主要 38 施設で構成する研究グループを組織し、日本公定書協会臨床研究データセンター、JCOG の臨床試験審査委員会や臨床試験支援機構の協力を得、適切なデザインの研究計画を作成、研究体制を整備した。平成14年9月に本試験の症例登録を開始した。症例集積は平成18年2月まで一次登録259例、二次登録233例となり、平成18年前半に症例登録が完了できる見込みである。計画より遅れているものの、ほぼ順調な症例集積ペースといえる。研究グループ活性化のための参加施設の入れ替え、近隣医療機関への紹介パンフレット配布などの症例集積推進策の成果ともいえる。定期モニタリングでは、予想どおりの腫瘍縮小効果が得られており、毒性についても予期した範囲の種類・程度・頻度で、現時点で安全性についても許容範囲と結論される。本研究は、ほぼ当初の計画通りに進められており、数年後には研究成果を公表できると考える。

E. 結論

限局期小細胞肺がんの予後改善を目指した「EP療法とAH-TRT同時併用後のEP療法とIP療法の第 III 相比較試験」は、研究グループ組織、データセンター、臨床試験支援機構など研究体制が整備され、適切なデザインの実施計画書を作成、第三者による公正な審査、承認を経て、平成14年9月に症例登録を開始した。

平成18年2月までに、259症例が登録されており、ほぼ順調な症例集積が進められている。定期モニタリングでは、主たる毒性は、好中球減少、食欲不振、発熱、感染など予期されたものであり、安全性についても十分耐容可能であることが確認された。

F. 健康危険情報

なし

G. 研究発表

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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

研究成果の刊行に関する一覧表

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局所限局小細胞肺がんの集学的治療に関する研究

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Phase I pharmacokinetic and pharmacogenomic study of E7070 administered once every 21 days

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E7070 is a novel sulfonamide anticancer agent that disrupts the G1/S phase of the cell cycle. The objectives of this phase I study of E7070 were to estimate the maximal tolerated dose (MTD), to determine the recommended dose for phase II, and to clarify the pharmacokinetic profile of E7070 and its relation to polymorphisms of CYP2C9 (*2, *3) and CYP2C19 (*2, *3) in Japanese patients. Patients received 1–2-h i.v. infusions of E7070 (400, 600, 700, 800 or 900 mg/m²) on day 1 of a 21-day cycle. Twenty-one patients received between one and eight cycles of E7070. The dose-limiting toxicities (DLT) comprised leukopenia, neutropenia, thrombocytopenia, elevation of aspartate aminotransferase, colitis, and ileus. The mean area under the plasma concentration–time curve (AUC) for successive dose levels increased in a non-dose-proportional manner. Two patients were heterozygous for the CYP2C9 mutation. For CYP2C19, eight patients were wild type and the remainder had heterozygous (n = 8) or homozygous mutations (n = 5). Regarding the CYP2C19 genotype, the AUC of patients with mutant alleles were higher than those of patients with wild type at a dose of 600 mg/m² or more. The severity of toxic effects, such as myelosuppression, seemed to depend on the AUC. No partial responses were observed. One patient treated at a dose of 700 mg/m² experienced a maximum tumor volume reduction of 22.5%. The MTD was estimated to be 900 mg/m². A dose of 800 mg/m² is recommended for further phase II studies. The pharmacokinetic/pharmacodynamic properties of E7070 seemed to be influenced by CYP2C19 genotype. The observed safety profile and preliminary evidence of antitumor activity warrant further investigation of this drug in monotherapy or in combination chemotherapy. (*Cancer Sci* 2005; 96: 721–728)

E7070, *N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide, has been reported to exhibit a potent antitumor activity by blocking cell cycle progression. *In vitro* studies indicate that the drug disrupts the G_1/S phase, thereby inducing cell cycle arrest and apoptosis.[1] Although E7070 is not a direct inhibitor of cyclin-dependent kinases (CDK), it causes depletion of cyclin E, with a reduction in CDK2 catalytic activity.[2] The exact mechanism of cyclin E/CDK2 inactivation is unclear. Transcriptional repression of cyclin H in a p53-independent manner also occurs in response to E7070.[3] The reduction in G_1 CDK activity induces arrest at the G_1/S boundary, accompanied by hypophosphorylation of retinoblastoma (Rb) protein and decreases in CDK2, cyclin A, and cyclin B proteins.[1] E7070 activity is associated with upregulation of p53 and p21, which may contribute to the reduced Rb phosphorylation, as well as subsequent apoptosis. In preclinical models, E7070 was cytotoxic to human HCT116 colon carcinoma and LX-1. E7070 exhibits more potent *in vivo* antitumor effects than 5-fluorouracil, mitomycin C, and irinotecan.[4]

E7070 has been the subject of four clinical phase I studies. In the first trial, E7070 was administered once every 21 days at doses between 50 and 1000 mg/m²,[5] and in the second trial E7070 was administered five times per day once every 3 weeks at doses between 10 and 200 mg/m² per day.[6] Other schedules used were a weekly infusion given over 4 consecutive weeks repeated every 6 weeks,[7] and a continuous intravenous infusion for 5 days every 3 weeks.[8] In the single-dose every 3 weeks study, neutropenia and thrombocytopenia were dose-limiting at 700 and 800 mg/m².[5] In the second study, neutropenia and thrombocytopenia were dose-limiting at 160 and 200 mg/m².[6] In the study that used a weekly dose schedule, neutropenia and thrombocytopenia were also dose-limiting toxicities (DLT) and other DLT included stomatitis, diarrhea, nausea, and fatigue.[7] Partial responses were observed in patients with breast and endometrial cancer.[6,7]

During a phase I trial, three patients receiving prophylactic daily oral maintenance therapy with acenocoumarol developed a hemorrhagic tendency and/or a prolonged prothrombin time following treatment with 700 and 800 mg/m² of E7070.[5] The major metabolic enzyme for acenocoumarol is cytochrome P450 (CYP)2C9.[9] *In vitro* studies have shown that E7070 has the potential to inhibit CYP2C9 and CYP2C19.[10] The pharmacokinetic drug–drug interaction study indicated that primary interaction of the two drugs could occur via inhibition by E7070 of acenocoumarol metabolism.

Based on these results from the previous phase I and pharmacokinetic trials, the present phase I study was designed to evaluate ascending doses of E7070 administered as a single dose by 1–2-h i.v. infusion every 21 days. The objectives of the study were to determine the maximum tolerated dose (MTD) and the dose to be recommended for use in future phase II studies, as well as to assess the safety, pharmacokinetic profile and preliminary antitumor activity of the drug. We also evaluated the influence of genetic polymorphisms of CYP2C9 and CYP2C19 on the pharmacokinetics of E7070.

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Materials and Methods

Patient selection

Japanese patients with histologically or cytologically confirmed malignant solid tumors refractory to conventional chemotherapy, or tumors for which no effective therapy was available, were candidates for this study. Eligibility criteria included the following: age between 20 and 75 years; World Health Organization (WHO) performance status 0–1, life expectancy ≥ 3 months, absolute leukocyte count ≥ 4000/μL and < 12 000/μL, absolute neutrophil count ≥ 2000/μL, hemoglobin level ≥ 9 g/dL, platelet count ≥ 100 000/μL, serum creatinine level <1.5 mg/dL or creatinine clearance ≥ 50 mL/min, and arterial partial pressure of oxygen of 65 torr or more. Additional entry criteria were serum bilirubin ≤ 1.5 mg/dL, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 100 IU/L. Before study entry, a 6-week interval was required for patients previously treated with mitomycin C or nitrosoureas, a 4-week interval was required for other chemotherapy, endocrinotherapy, surgery, radiation therapy, immunotherapy treatments or other investigational agents, and a 2-week interval after blood transfusion or administration of granulocyte-colony stimulating factor (G-CSF). Patients were ineligible for the study if they had symptomatic central nervous system metastases, active infection, or other non-malignant disease, which was considered to be incompatible with the protocol. Patients who were receiving corticosteroids or coumarin anticoagulants less than 2 weeks prior to administration of E7070 were excluded from the study. The protocol was approved by the institutional review boards of the National Cancer Center, and all patients gave written informed consent prior to study entry.

Dosage and dose escalation

E7070 was provided as a lyophilized powder in 500-mg vials by Eisai Co. (Tokyo, Japan). The starting dose of E7070 was set at 400 mg/m^2 because only mild to moderate grade 1 to grade 2 toxicity was observed at doses of 600 mg/m^2 or lower in the previous phase I study.[5] Subsequent doses were to be escalated to 600, 700, 800 and 900 mg/m^2. E7070 was dissolved in 20 mL of distilled water, then added to 500 mL of normal saline for injection, and this solution was administered by intravenous infusion over 1 h at doses of 400 and 600 mg/m^2. Injection site reaction was observed in one of three patients at 400 mg/m^2 and three of three patients at 600 mg/m^2. Therefore, E7070 was given in 1000 mL of normal saline over 2 h at 700, 800, and 900 mg/m^2. Patients were hospitalized for the first course of E7070 and remained hospitalized for close observation for 21 days thereafter. Subsequent courses could be administered on an outpatient basis with weekly patient evaluations by the investigator.

Patients were enrolled in cohorts of three patients per dose level and observed for 21 days; the observation period was extended to 42 days if a longer recovery period was needed. If one of the three patients experienced DLT, then three additional patients were treated at the same dose. If two or more of three or six patients experienced DLT, that dose level was regarded as the MTD. If none of the first three patients demonstrated DLT, then the next three patients were treated at the next (higher) dose level. Individual patients who did not demonstrate DLT and showed no evidence of disease progression could receive E7070 at the originally assigned dose.

After the MTD had been determined, a dose below the MTD was evaluated in a total of six patients for identification of the proposed recommended dose for phase II study. The recommended dose was the highest dose at which less than 33.3% of treated patients experienced DLT.

Definition of dose-limiting toxicity

The DLT was defined as the occurrence of any of the following events during cycle 1 that were attributable to E7070: National Cancer Institute Common Toxicity Criteria (NCI CTC) (version 2.0) grade 3 or 4 non-hematological toxicity (except nausea, vomiting effectively managed with symptomatic treatment, or alopecia), grade 4 leukopenia, grade 4 neutropenia accompanied by fever of ≥ 38.5°C, or that persisted ≥ 5 days, and platelet count < 25 000/μL. Prophylactic use of colony-stimulating factors was not permitted during the first cycle; however, patients who had neutropenia that had met the criteria for DLT were permitted to receive concomitant treatment with G-CSF.

Evaluation of patients

Safety was evaluated on the basis of physical findings, vital signs, adverse events, and laboratory parameters obtained at baseline and periodically throughout the study. During the first cycle, hematology studies were performed at least twice a week, while vital signs, physical examinations (including evaluation of performance status) and serum biochemistry were measured on days 1, 8 and 15. Toxicity evaluations of subjective and objective findings were performed according to the NCI CTC (version 2.0) on days 1, 8 and 15. For the second and subsequent cycles, vital signs, laboratory tests and toxicity evaluations based on subjective and objective findings were performed on days 1, 8, and 15 of each cycle. Blood glucose was monitored before the dose of E7070 and after the end of infusion. Blood coagulation studies were carried out before each dose for all cycles and also at day 8 of the first cycle. Efficacy was assessed by the physician on the basis of antitumor effect according to the Response Evaluation Criteria in Solid Tumors (RECIST).[11] If an antitumor effect was observed, the disease site was re-evaluated at least 4 weeks later to confirm the response.

Pharmacokinetics

Pharmacokinetic studies were performed during the first cycle of treatment. On day 1, blood samples (4 mL each) were drawn from an indwelling intravenous cannula in the arm contralateral to that bearing the infusion line. Samples were collected in heparinized tubes, preinfusion, at 30 min after the start of the infusion, at the end of the 1- or 2-h infusion, and at 10, 30, and 60 min and 2, 4, 6, 10, 24, 48, 72, 96, 168, and 240 h after the infusion. The samples were centrifuged at 1500×*g* for 10 min at 5°C, and the resulting plasma samples were stored at −20°C until analysis. Urine samples were collected before the start of E7070 infusion and over three 24-h intervals for 72 h after the start of the infusion. The concentrations of E7070 in plasma and in urine were analyzed at Eisai Co. by means of validated high-performance

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liquid chromatography methods with UV detection (HPLC-UV). The lower limit of quantification was 20 ng/mL. *N*-(3-Chloro-7-indolyl)-4-(*N*-methylsulfamoyl)benzenesulfonamide (ER-67771)[12] was used as an internal standard.

Plasma, the internal standard and 0.1 mol/L phosphate buffer (pH 6.8) were vortexed. After adding ethyl acetate, the mixture was shaken and centrifuged. The organic layer was collected and transferred into a glass tube, then evaporated under nitrogen flow in a drying block. The residue was dissolved in CH_3CN-6.7 mmol/L phosphate buffer (pH 6.6), and the solution was injected into an HPLC apparatus.

Chromatographic separation was achieved by using a column switching method with Asahipak C8P-50 (Showa Denko, Tokyo, Japan) as a separation column and YMC-pack ODS-AM-312 (YMC) as an analytical column. Mobile phases were CH_3CN: 6.7 mmol/L phosphate buffer (pH 6.6; 360:640 [v:v]) for separation and CH_3CN: 6.7 mmol/L phosphate buffer (pH 7.4; 360:640 [v:v]) for analysis. E7070 was monitored by UV detection at 280 nm.

Pharmacokinetic parameters of E7070 after a single dose administration during the first cycle were determined by non-compartmental analysis using WinNONLIN (Pharsight Corporation, CA, USA). The apparent elimination rate constant at the terminal phase (λ_z) was estimated by linear regression analysis from the terminal log-linear declining phase to the last quantifiable concentration. The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/\lambda_z$. The area under the plasma concentration–time curve from zero to the last quantifiable sampling time, $AUC_{(0-tn)}$, was obtained by the log trapezoidal rule. The AUC from zero to infinity was calculated as $AUC_{(0-tn)} + C_n/\lambda_z$, where C_n was the last quantifiable concentration. The clearance was calculated as dose/AUC. The mean residence time (MRT) was estimated from AUMC/AUC, where AUMC is the first moment curve. The volume of distribution was calculated as MRT × clearance.

Genotyping procedures for CYP2C9 and CYP2C19

Genotyping was conducted using the Invader assay (BML, Tokyo, Japan). Genomic DNA was isolated from whole blood with the QIAamp DNA Blood Kit (Qiagen, CA, USA). The primary probes (wild type and mutant probes) were used to detect C430T (*2) and A1075C (*3) mutations of CYP2C9, and G681A (*2) and G636A (*3) of CYP2C19, respectively. The invader assay fluorescent resonance energy transfer (FRET)-detection 96-well plates (Third Wave Technologies, WI, USA) contained Cleavase enzyme, FRET probes, MOPS buffer and polyethylene glycol. Eight microliters of mixtures consisting of an appropriate primary probe, Invader oligonucleotide and $MgCl_2$ was added to the wells, followed by addition of 7 μL of the heat-denatured genomic DNA, and this was overlaid with 15 μL of mineral oil. For only CYP2C9*2 (C430T) detection, a dilution of the CYP2C9-specific polymerase chain reaction (PCR) product was used instead of genomic DNA, because the CYP2C9*2 (C430T) detection point has the same sequence on CYP2C19. The plates were incubated at 63°C for 4 h for genomic DNA or 1 h for the PCR product. The fluorescence intensities were measured on a Cytofluor 4000 fluorescence plate reader (Applied Biosystems, CA, USA) with excitation at 485/20 nm (wavelength/bandwidth) and emission at 530/25 nm for FAM dye detection, and excitation at 560/20 nm and emission at 620/40 nm for RED dye detection.

Subjects having either the *2 or *3 allele (*1/*2 or *1/*3) were defined as hetero extensive metabolizers (hetero EM), those with two mutated alleles (*2/*2, *2/*3 or *3/*3) as poor metabolizers (PM), and those with no mutated alleles (*1/*1) as homo EM.

Table 1. Patients' characteristics

No. entered	21
Age (years)	
Median	57
Range	35–70
Male:female (no. patients)	15:6
WHO performance status (no. patients)	
0	7
1	14
Tumor type (no. of patients)	
Colorectal	15
SCLC	2
Gastric	1
NSCLC	1
Liposarcoma	1
Mesothelioma	1
Prior treatment	
Chemotherapy	
No. prior regimens (no. patients)	
0	0
1	1
2	5
>3	15
Surgery (no. patients)	18
Radiation therapy (no. patients)	7

SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; WHO, World Health Organization.

Results

Patients' characteristics

Twenty-one patients (15 male and six female) were enrolled into the study (Table 1). All patients had a good performance status and had received previous chemotherapy regimens. The colon/rectum was the most commonly noted primary disease site. All patients were evaluable for toxicity during the first cycle, and for efficacy. Twenty-one patients received 42 cycles of treatment. The median number of cycles administered per patient was one (range, 1–8).

Dose escalation and identification of DLT, MTD, and the recommended phase II dose

The DLT in this study were leukopenia, neutropenia, thrombocytopenia, elevation of AST, colitis, and ileus. None of the patients treated at any dose of less than 800 mg/m^2 experienced DLT. At a dose of 900 mg/m^2, two of three patients experienced dose-limiting leukopenia, neutropenia, and thrombocytopenia, identifying this dose level as the MTD. At the same dose, grade 3 colitis and grade 3 AST elevation were observed in one patient. Therefore, three additional patients were enrolled at 800 mg/m^2. One of the additional three patients evaluated for safety at 800 mg/m^2 experienced