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Effectiveness of omega-3 fatty acid administration on completion rate of adjuvant chemotherapy for biliary tract cancer: study protocol for a single-center, open-label, single-arm, historically controlled study

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ABSTRACT

Introduction: Multimodal treatment prolongs the survival of biliary tract cancer patients. However, there are not so many choices of chemotherapy, and to complete each chemotherapy session is important. Adjuvant chemotherapy has been tried for biliary tract cancer, but the completion rate of 75% this is not enough. Body weight loss and cholangitis are reasons for interruption of chemotherapy. Previous reports suggested that nutritional intervention containing omega-3 fatty acid maintained bodyweight and improved the completion rate of chemotherapy. Moreover, omega-3 fatty acid has an anti- inflammatory effect. Omega-3 fatty acid is expected to improve the completion rate of adjuvant chemotherapy in biliary tract cancer patients. The aim of this study is to evaluate the effectiveness of omega-3 fatty acid for patients planning adjuvant chemotherapy for biliary tract cancer.

Method and analysis: This study is a single center, open-label, single-arm, historically controlled study with a planned enrollment of 55 participants. Protocol treatment consists of four courses of S-1 adjuvant chemotherapy and oral omega-3 fatty acid pharmaceutic adjuvant (LOTRIGA 2g® (Takeda pharmaceutical Co. Ltd)) which include 2g of omega-3 fatty acid from day one until day168 of the treatment period. The primary endpoint is the completion rate of four total courses of S-1. The secondary endpoints are post-operative

cholangitis, time to recurrence or distant metastasis, changes of nutritional index, changes of lymphocyte blast transformation test induced by phytohemagglutinin, and comcanavalin A and diamine oxidase serum activity during adjuvant chemotherapy. All adverse events will be evaluated.

Ethics and dissemination: This protocol was approved by the Institutional Review Board of Kobe University Hospital. The findings from this study will be presented at national and international conferences and published in peer-reviewed journals.

Trial registration number: UMIN Clinical Trials Registry UMIN000031247.

ARTICLE SUMMARY

Strengths and limitations of this study

This study investigates the effectiveness of omega-3 fatty acid on the completion rate of adjuvant chemotherapy for biliary tract cancer.

This study is well planned for the selection of participants because biliary tract cancer include four different cancers depends on its' location.

A main limitation of this study is related to it being a historically controlled study, which includes some bias.

Because the primary endpoint of this study is the completion rate of chemotherapy, not the prognosis of patients, the effect on survival can only be interpreted as a guide.

INTRODUCTION

While surgical resection is a definitive treatment for biliary tract cancer, the 5-year survival rate is about 30-50% [1], which is lower than that of other gastrointestinal cancers. Because of the limitation of surgical resection, multimodal treatment is expected to improve rates of survival [2]. In spite of the importance of adjuvant chemotherapy, its start and continuation is difficult after biliary surgery. This means that biliary surgery such as liver resection and pancreaticoduodenectomy is very invasive, and easily results in delayed recovery from surgery as well as bacterial translocation which arises from malnutrition and immunological deterioration. Uncontrolled bacterial translocation causes cholangitis, and cholangitis interrupts chemotherapy. A feasibility study of S-1 adjuvant chemotherapy for biliary tract cancer showed that 75.8% of biliary tract cancer patients completed 24 weeks' protocol [3]. The main causes of discontinuation were adverse events and detection of relapse. This rate is similar to that for gastric (78%) [4] and pancreatic (72%) cancers [5]. Biliary tract cancer has only a few choises of chemotherapy; therefore completing each chemotherapy session is required. Improvement in the completion rate of adjuvant chemotherapy is an important result for biliary tract cancer.

Previous reports suggested that body weight loss is one cause of interruption of adjuvant chemotherapy [6]. Nutritional intervention containing omega-3 fatty acid maintained

body weight [7 8] and improved the completion rate of adjuvant chemotherapy[7]. Omega-3 fatty acid are metabolized to lipid mediators, and these mediators evoke anti-inflammatory and novel pro-resolving mechanisms as well as enhance microbial clearance [9]. In addition, enteral immunonutrition using omega-3 fatty acid reduces bacterial translocation and atrophy of intestinal mucosal villi in rats with obstructive jaundice. Omega-3 fatty acid are expected to be a nutritional intervention for patients after biliary surgery in terms of maintenance of body weight and immunity. Moreover, as previous reports suggested an anti-tumor effect of omega-3 fatty acid [10-13], this is an appropriate drug for cancer patients.

The aim of this study is to evaluate the efficacy of omega-3 fatty acid on adjuvant chemotherapy. Because patients to whom chemotherapy was administered tend to reduce dietary intake, and patients after biliary surgery are prone to cholangitis, omega-3 fatty acid is used in this study for nutritional support and anti-inflammatory treatment. We conduct this study with the estimation that omega-3 fatty acid improves tolerance for adjuvant chemotherapy after biliary surgery from the point of view of body weight and anti-cholangitis.

METHODS AND ANALYSIS

Study design

This study is a single-center, open-label, single-arm, historically controlled study of patients

who are administered S-1 after surgical resection of biliary tract cancer. The aim of this study is to examine the improvement of completion rates of adjuvant chemotherapy by administration of omega-3 fatty acid. All participants are scheduled to receive administration of four cycles of S-1 adjuvant chemotherapy, and oral omega-3 fatty acid during chemotherapy (Figure1). This study includes a 3-year period of registration and 1 year of follow-up. This study protocol follows the SPIRIT-statement.

Sample size

The sample size was calculated based on the primary endpoint. This study is planned for 55 participants. Previous study reported that the completion percentage of four courses of S-1 chemotherapy was 76% in biliary tract cancer patients[3]. The reasons for discontinuance were adverse events (12%) and early recurrence (12%). We set 0.76 as the threshold proportion. Aoyama *et al* reported that administration of nutritional support with omega-3 fatty acid caused a 100% completion rate of adjuvant chemotherapy in gastric cancer[6]. We set 0.9 as the estimated proportion for our study because biliary tract cancer has about 10% early recurrence.

Under the estimation of 0.76 for threshold proportion and 0.9 for estimated proportion, 51 participants are required for one-sided significance level 0.025 and power 1-0.2. There is a potential for a few dropouts from follow-up, so we plan for a total of 55 participants.

There are about 25 target surgical operations in our institution per year, so we set a registration period of 3 years.

Study participants

Potential participants are all patients on whom surgical resection of biliary tract cancer is performed during the study period. After surgical resection, participants are recruited for the study, and they must provide written, informed consent before enrolling in the study. Participants who satisfy all the inclusion criteria and none of the exclusion criteria (Table1) are enrolled in this study.

Schedule of the study

Table2 shows the schedule of the study. After written informed consent, researchers perform a screening test and check inclusion and exclusion criteria. If participants meet all eligibility criteria, they are registered in the study. No protocol treatment before registration and no revocation after registration are permitted. After all data are entered and registration numbers given, then patients are counted as registered. Participants undergo protocol treatment within 14 days after registration.

After registration this study consists of a maximum two-week pre-observation period, 24-week treatment period, and four-week post-observation period. During the pre-

observation period, participants taking an antilipemic agent change their drug to omega-3 fatty acid formulations.

Participants do not receive any anti-cancer treatment after protocol treatment until the recurrence of cancer.

Intervention

S-1

Participants receive S-1 orally with the amount calculated considering body surface area and creatinine clearance (Table3). Body weight for this calculation is that at registration. They receive S-1 twice daily for 28 consecutive days, and the drug is withdrawn for the next 14 days (one cycle). This administration of S-1 is repeated every six weeks for up to four cycles. If a cycle ends within 42 days for some reason, participants undergo an additional cycle until the end of the 24-week treatment period. A cycle is not newly started after a treatment period, and ongoing cycles on day 168 of the treatment period continue to the end.

Participants should meet the following criteria when starting the treatment from the second cycle: 1) ECOG performance status is 0, 1, or 2; 2) neutrophil count is at 1000 cells/μL or higher; 3) platelet count is at 50000 cells /μL or higher; 4) AST concentration is three times the upper limit or lower; 5) ALT concentration is three times the upper limit or lower; 6) T-Bil concentration is 1.5 times the upper limit or lower; 7) percutaneous arterial

oxygen saturation is 90 % or more, and respiratory status dose is not worse than at registration; and 8) researchers consider the treatment can be performed safely.

Omega-3 fatty acid formulations

All participants take LOTRIGA 2g® (Takeda pharmaceutical Co. Ltd) which include 2g of omega-3 fatty acid from day one to day168 of the treatment period.

Withdrawal and reduction of the drug

If any of adverse events are observed, researchers should withdraw S-1 and omega-3 fatty acid formulations. When participants meet the criteria for restart within seven days, they restart S-1 as the same cycle. In this case, the next cycle can start from the scheduled day with at least a seven-day interval. When participants do not meet the criteria for restart after more than 8 days, the cycle stops at that time. If participants meet the starting criteria, they can start a new cycle. When participants do not meet the restart criteria after more than 42 days since the last day of taking S-1, they should stop the protocol treatment.

When restarting S-1 after withdrawal due to an adverse event, participants get a reduced dose of S-1. The daily dose of S-1 is reduced from 120 mg to 100mg, from 100 mg to 80 mg, from 80 mg to 60mg, or from 60mg to 50mg. Participants have two opportunities for reducing the drug. Participants taking 50mg of S-1 should stop the protocol treatment.

If participants develop febrile neutropenia, they should be evaluated for t infectious status, and antimicrobial agent or granulocyte colony stimulating factor (G-CSF) is used.

Adherence

Researchers provide patient compliance instructions, and confirm at each visit the remnant of drugs and whether participants have ingested the correct dose of S-1.

Concomitant drugs and therapy

There is no concomitant drugs and therapy during this study.

Outcomes

Primary outcome

The primary outcome is the completion rate of adjuvant chemotherapy. Completion is defined as participants who can finish a total of four cycles of S-1 adjuvant chemotherapy, regardless of relative performance index (RP: proportion of actual dose per expected dose) after surgical resection of biliary tract cancer.

Secondary outcome

Secondary outcomes are as follows:

1) Post-operative cholangitis
2) Time to recurrence or distant metastasis
3) Changes of nutritional index from registration until the completion of chemotherapy
4) Changes of lymphocyte blast transformation test induced by phytohemagglutinin (PHA) and comcanavalin A (Con-A) during adjuvant chemotherapy

5) Change of diamine oxidase serum activity (DAO) during adjuvant chemotherapy

6) All adverse events after medication. A modified version of The Common Terminology Criteria for Adverse Events (CTCAE v4.0) is used to grade adverse events.

Prospective adverse events are as follows: coagulation disorder, lipid metabolism disorder, abnormality of laboratory data, fatigue, fever, gastrointestinal disorders, skin and subcutaneous tissue disorders, metabolism and nutrition disorders, nervous system disorders, blood and lymphatic system disorders, eye disorders, infections and infestations, injury and procedural complications.

Data analysis

Participants who are recruited into this study and receive administration of one of the test drugs at least once are included in the full analysis set (FAS). Participants who cannot provide baseline data and have serious violations of protocol are not included in the FAS. Per protocol set (PPS) is defined as participants in the FAS who comply with exclusion criteria, concomitant drugs, and concomitant therapy. The analysis of FAS is performed for each outcome. The safety is analyzed in PPS. All analysis is performed with fixed data.

Primary outcome

The point estimate of the completion rate of S-1 and 95% confidence interval (CI) is

calculated. Participants who complete S-1 are defined as participants who have performed four scheduled cycles of S-1, or have continued treatment for 168 days.

Secondary outcomes

Analyses of secondary outcomes take the form of simple descriptive statistics (e.g., proportions and IQRs, means and SDs) and where appropriate, point estimates of effect sizes (e.g., mean differences) and associated 95% CIs. The recurrence-free survival curves iscalculated using the Kaplan-Meier method, and 95% CIs are calculated.

Safety analysis

For the evaluation of the safety of the study, all adverse events due to the study are counted. Severe adverse events are defined as the following three events: Grade 4 of non-blood system disorder, early mortality within 30 days after the last treatment, or mortality causally related to the protocol treatment.

Interim analysis is be performed in this study. All statistical analyses are conducted using JMP software (version 13.0, SAS, Cray, NC, USA).

Data management

Researchers make case report forms (CRF) for each participant. Modification of CRF is

permitted only when it does not exceed the prescribed range and is not a burden to participants. The principal investigator confirms and signs the CRF. CRFs are made at registration (within two weeks), treatment period (each two cycles), end of treatment (within 30 days), and in the follow-up period.

Data and safety monitoring

Independent data and safety monitoring is conducted. The following materials are reviewed every six months: informed consent obtained and signed, participant retention, study implementation system, study safety and data, and study progress.

Confidentiality

All study-related information are stored securely at the study site and identified by a coded number only to maintain participant confidentiality. All participant information are stored in locked file cabinets in areas with limited access. All records that contain names or other personal identifiers are stored separately from study records identified by code number. All local databases are secured with password-protected access systems. Participants' study information are not be released outside of the study without the written permission of the participant.

ETHICS AND DISSEMINATION

This study is conducted according to the declaration of Helsinki, Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan, and Management Guidelines of Conflict of Interest in our institution. The protocol has been approved by the Institutional Review Board of Kobe University Hospital (No 290086).

Researchers explain the study to potential participants with information sheets. Patients are given the opportunity to ask questions and have enough time to consent to the study. Researchers obtain written consent from patients willing to participate in the study. Information sheets and consent forms are provided for all participants involved in the study.

The result of this study will be disseminated through academic conferences and peer-reviewed journals. All authors will review and approve the paper before publication. If there are several papers related to this study, the investigator will appoint each author.

DISCUSSION

The biliary tract is divided into four anatomical component ; they are intrahepatic bile duct, extrahepatic bile duct, gallbladder, and ampulla of vater. Cancer staging is different in each origin, and the prognosis is also different. To select proper patients for this study, we exclude patients of Stage I of perihilar cholangiocarcinoma and gallbladder cancer and Stage IA of cancer ampulla of vater and distal bile duct cancer. That is because these Stage IA or Stage I

patients have a better prognosis compared to patients with other stages. The five-year survival rate of Stage I gallbladder cancer patients and Stage IA ampulla of vater cancer patients are 87.5 % and 82.9 %, respectively. Although cancer of Stage I (five-year survival rate is 69.8%) perihilar cholangiocarcinoma and Stage IA (59.5%) distal biliary tract cancer patients have worse prognosis than gallbladder cancer and cancer of ampulla of vater patients, cancer death is not common. Potential reasons for poor prognosis are perioperative death and late complications like cholangitis. These patients get few benefits from adjuvant chemotherapy. On the other hand, the five-year survival rate of Stage I intrahapetic cholangiocatcinoma patients is 36.3 % which is worse than the same stage of other biliary tract cancers. Adjuvant chemotherapy seems to be beneficial for patients of Stage I intrahepatic cholangiocarcinoma. Though the target for adjuvant chemotherapy for biliary tract cancer is still controversial, we have selected the relatively poor-prognosis group in biliary tract cancer. Moreover, to align the patients post-surgical background, we have selected the type of surgical operation which includes biliary reconstruction.

The most common reason for interruption of adjuvant chemotherapy was an adverse event in biliary tract cancer [3]. Seo et al [14] reported that hypoalbuminemia (<3.5mg/dL) was associated with adverse events greater than grade 3 in gastric cancer. Hypoalbuminemia is also one risk factor for interruption of adjuvant chemotherapy in pancreatic cancer patients [15]. The albumin level is one of the indications of nutritional status. According to a previous

study, body weight loss is associated with the continuance of adjutant chemotherapy [6], and nutritional intervention including omega-3 fatty acid has an effect on several cancers [7 8].

It is well known that chemotherapy including 5-fuluorouracil(5-FU) causes gastrointestinal injury[16]. 5-FU also showed toxicity to helper T cells in mice, which seems to cause immunesuppression[17]. These changes might result in bacterial translocation(BT)[18]. From the viewpoint of immunity and infection, omega-3 fatty acid plays a preventive role. Matsunaga H et al. reported that a diet containing omega-3 fatty acid suppresses the thickening of mucous, submucosa, and the muscular layer due to inflammation in mice compared with the control diet[19]. Moreover omega-3 fatty acid inhibits infiltration of inflammatory cells into the muscularis mucosa[19]. Moreover, the rate of bacterial translocation and ileal change in pre- and postoperative feeding with omega-3 fatty acid in rats on which were performed common bile duct ligation were the same as that of the control group, while bacterial translocation and atrophy of intestinal mucosa in postoperative feeding group increased compared to the control group[20]. From these reports, it is thought that the administration of omega-3 fatty acid from the start of chemotherapy when intestinal mucosal disorder is not yet occurring can keep intestinal mucosa normal and prevent intestinal inflammation and the interruption of anticancer agent by infection.

In addition to the nutritional aspect, omega-3 fatty acid has an anti-inflammatory effect. Post biliary reconstruction, it is easy for patients to experience cholangitis and stop

chemotherapy. Omega-3 fatty acid is known to calm acute inflammation by regulation of inflammatory cytokines [9]. Furthermore, in vivo experiments showed suppression of the proliferation of cancer cells by inducing apoptosis in several cancers [10 12 13].

In this study we expect that omega-3 fatty acid will improve the completion rate of adjuvant chemotherapy and will result in a good prognosis in biliary tract cancer patients.

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Registry This study is registered at UMIN Clinical Trials Registry (UMIN000031247) and Japan Registry of Clinical Trials (jRCTs051180007):.

Registration Data Set: https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000035677

Registration Data Set: https://jrct.niph.go.jp/detail/349

Contributors All the authors contributed to the study design and writing and revising the protocol. All authors read and approved the final manuscript.

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Competing interests None declared.

Patient consent Obtained

Patient and Public Involvement We did not involve patients or the public in our work

Ethics approval Kobe University Clinical Research Ethical Committee

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Protocol amendment number: 1.4

Table1 Inclusion and exclusion criteria

Inclusion criteria
1) pathologically diagnosed as adenocarcinoma (in case of combined cancer, adenocarcinoma is dominant) 2) UICC stage is as follows: - any T, N0 or N1 and M0 in intrahepatic cholangiocarcinoma - T2, 3, 4, N0, and M0 in extrahepatic cholangiocarcinoma - any T, N1 and M0 in extrahepatic cholangiocarcinoma 3) age 20 - 80 years or younger 4) ECOG perfomance status is 0 or 1 5) CT and MRI show no metastases and moderate or less pleural effusion* or ascites** 6) Performed pancreaticoduodenectomy, hepatectomy, bile duct resection, caudal lobectomy and/or cholecystectomy, with D1 or more extensive lymphadenectomy*** 7) No history of any prior chemotherapy or radiation therapy 8) 14 to 70 days from surgical resection 9) Good oral intake 10) No watery diarrhea meets all the following laboratory data values within 14 days before registration a) neutrophil ≥ 1,200/mm3 b) platelet ≥ 10,000/mm3 c) hemoglobin ≥ 8.0g/L d) total bilirubin ≤ 2.0 mg/dL e) AST ≤100IU/L f) ALT ≤ 100IU/L g) serum creatinine ≤ 1.2mg/dL h) creatinine clearance (CC) ≥ 40 mL/min (CC calculated by Cockcroft-Gault Equation) 11) Written informed consent
Exclusion criteria
1) Allergic predisposition or drug hypersensitivity 2) Double or multiple cancer diagnosed or treated in the past five years. (except for carcinoma in situ) 3) Active infectious disease (except for virus hepatitis) 4) Fver above 38 degrees C 5) Current pregnancy, breastfeeding, planning to become pregnant during the study 6) Psychological disease and/or symptoms

7) Severe liver or renal dysfunction
8) Uncontrollable hypertension or diabetes mellitus
9) Uncontrollable ischemic heart disease:
   - angina developed or worsening within three weeks
   - myocardial infarction developed within six months
10) Taking flucytosine, phenytoin, or warfarin potassium
11) Interstitial pneumonitis, pulmonary fibrosis, and pulmonary emphysema
12) Taking omega-3 fatty acid formulations
13) Considered unsuitable by their attending physician

CT computed tomography MRI magnetic resonance imaging

*Moderate plural effusion is defined as effusion occupying one third of the lung field by chest X-ray. ** Moderate ascites is defined as ascites within the pelvic cavity. ***In case of intrahahepatic cholangiocatcinoma without invasion to hepatic portal region, lymphadenectomy is not necessary.

Table2 Schedule of enrollment, interventions, and assessments.

	STUDY PERIOD							
	pre-observation	**treatment period**						**post-observaton**
TIMEPOINT (week)	*14days before registration*	*0*	*2, 4, 6*	*9*	*12*	*15 ,18, 21*	*Close-out, 24*	*28*
VISIT	1	2	3, 4, 5	6	7	8, 9, 10	11	12
ENROLLMENT:								
Eligibility screen	X							
Informed consent	X							
Background Performance status Blood test	X							
INTERVENTIONS:								
S-1		←	—	—	—	—	→	
LOTRIGA		←	—	—	—	—	→	
ASSESSMENTS:								
Symptoms			X	X	X	X	X	X
Body weight			X	X	X	X	X	X
General status					X		X	X
Blood test			X	X	X	X	X	X
ConA, PHA, DAO	X				X		X	
Adverse event			X	X	X	X	X	X

Background includes age, gender, body weight, height, past history, complication, date of diagnosis, family history, medication, and operative information (e.g. date, method, blood loss,

operation time). Screening tests are general status, blood test, urinary test, computed tomography and electrocardiogram. General status includes blood pressure, body temperature, pulse rate and respiratory rate. Blood test includes complete blood count, differential count of leukocytes, total protein, albumin, total bilirubin, creatinine, aspartate transaminase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, Na, K, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride.

Table 3 dose of S-1

	dose of S-1	
body surface area (m^2)	50mL/min ≤ Ccr < 60mL/min	60mL/min ≤ Ccr
under 1.25	60mg/day	80mg/day
1.25 to 1.5	80mg/day	100mg/day
over 1.5	100mg/day	120mg/day

Figure legend

Figure1 Summary of study.

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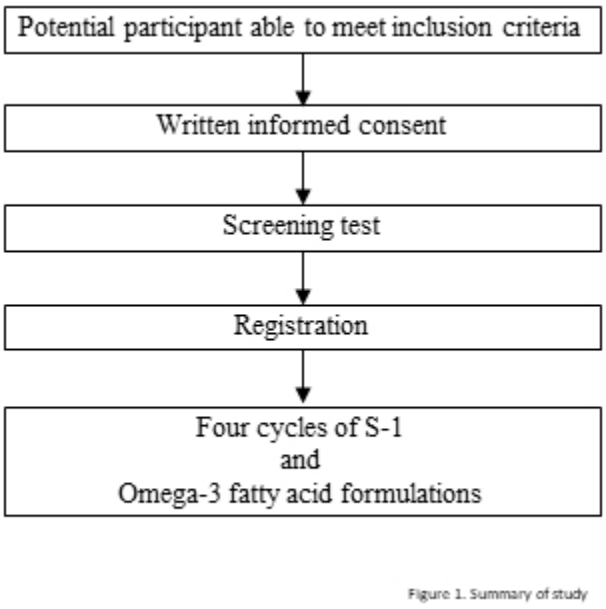

Figure 1. Summary of study

Figure legend
Figure1 Summary of study.

89x89mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	18
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	18
Protocol version	#3	Date and version identifier	18
Funding	#4	Sources and types of financial, material, and other support	18
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	18
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	N/A

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13-14
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	5-6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6-7
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8-9
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	9-10

Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-12
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA this study is single-arm
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA this study is single-arm
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA this study is single-arm

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA this study is single-arm
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA this study is single-arm
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13-14
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12-13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12-13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found,	14

		if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A not perform interim analysis
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
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Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A

Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	15
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A only in japanese
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Effectiveness of omega-3 fatty acid administration on completion rate of adjuvant chemotherapy for biliary tract cancer: study protocol for a single-center, open-label, single-arm, historically controlled study

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Effectiveness of omega-3 fatty acid administration on completion rate of adjuvant chemotherapy for biliary tract cancer: study protocol for a single-center, open-label, single-arm, historically controlled study

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ABSTRACT

Introduction: Multimodal treatment prolongs the survival of patients with biliary tract cancer (BTC). However, the chemotherapy choices for this disease are few, and completing each chemotherapy session is important. Adjuvant chemotherapy has been attempted for BTC, but has only had a 75% completion rate. Body weight loss and cholangitis are reasons for the interruption of chemotherapy. Previous reports suggested that nutritional intervention with omega-3 fatty acids maintained body weight and improved the completion rate for chemotherapy. Moreover, omega-3 fatty acids have an anti-inflammatory effect. Therefore, we theorized that omega-3 fatty acids would improve the completion rate of adjuvant chemotherapy in patients with BTC. The aim of this study is thus to evaluate the effectiveness of omega-3 fatty acids for patients planning adjuvant chemotherapy for BTC.

Method and analysis: This study is a single center, open-label, single-arm, historically controlled study with a planned enrollment of 55 participants. Protocol treatment consists of four courses of S-1 adjuvant chemotherapy and an oral omega-3 fatty acid pharmaceutic adjuvant (LOTRIGA 2g® [Takeda pharmaceutical Co. Ltd]), which includes 2 g of omega-3 fatty acids from day one until day 168 of the treatment period. The primary endpoint is the

completion rate of four total courses of S-1. Secondary endpoints are post-operative cholangitis, time to recurrence or distant metastasis, changes in nutritional index, changes in the lymphocyte blast transformation test induced by phytohemagglutinin, and concanavalin A and diamine oxidase serum activity during adjuvant chemotherapy. All adverse events will be evaluated.

Ethics and dissemination: This protocol was approved by the Institutional Review Board of Kobe University Hospital. The findings from this study will be presented at national and international conferences and published in peer-reviewed journals.

Trial registration number: UMIN Clinical Trials Registry UMIN000031247.

ARTICLE SUMMARY

Strengths and limitations of this study

This study investigates the effectiveness of omega-3 fatty acids on the completion rate of adjuvant chemotherapy for BTC.

This study is well planned for the selection of participants with BTC based on four different cancers depending on location.

A major limitation of this study is related to it being a historically controlled study, which includes some bias.

Because the primary endpoint of this study is the completion rate of chemotherapy, not the

prognosis of patients, the effect on survival can only be interpreted as a guide.

INTRODUCTION

While surgical resection is a definitive treatment for biliary tract cancer (BTC), the 5-year survival rate is about 30–50% [1], which is lower than that of other gastrointestinal cancers. Because of the limitation of surgical resection, multimodal treatment is expected to improve rates of survival [2]. In spite of the importance of adjuvant chemotherapy, the initiation and continuation of this in patients have proven difficult after biliary surgery. Biliary surgery, such as liver resection and pancreaticoduodenectomy, is very invasive, and easily results in delayed recovery as well as bacterial translocation (BT) that arises from malnutrition and immunological deterioration. Uncontrolled bacterial translocation causes cholangitis that interrupts chemotherapy. A feasibility study of S-1 adjuvant chemotherapy for BTC showed that 75.8% of patients completed a 24-week protocol [3]. The main causes of discontinuation were adverse events and relapse. This rate is similar to that for gastric (78%) [4] and pancreatic (72%) cancers [5]. Few chemotherapy choices for BTC exist and, therefore, the completion of each chemotherapy session is essential. It therefore follows that an improvement in the completion rate for adjuvant chemotherapy is important for the treatment of BTC.

Previous reports have suggested that body weight loss may be a reason for interruptions in adjuvant chemotherapy [6]. Nutritional intervention with omega-3 fatty acids

maintained body weight [7 8] and improved the completion rate of adjuvant chemotherapy [7]. Omega-3 fatty acids are metabolized to lipid mediators that evoke an anti-inflammatory response as well as novel pro-resolving mechanisms, and enhances microbial clearance [9]. In addition, enteral immunonutrition using omega-3 fatty acids reduces bacterial translocation and atrophy of intestinal mucosal villi in rats with obstructive jaundice. Omega-3 fatty acids are expected to be a nutritional intervention for patients after biliary surgery in terms of the maintenance of body weight and immunity. Moreover, since previous reports have suggested an anti-tumor effect for omega-3 fatty acids [10-13], this is an appropriate drug for cancer patients.

The aim of this study is to evaluate the efficacy of omega-3 fatty acids on adjuvant chemotherapy. Because patients to whom chemotherapy was administered tended to reduce their dietary intake, and those after biliary surgery are prone to cholangitis, omega-3 fatty acids are used in this study for nutritional support and as anti-inflammatory treatment. We will conduct this study with the hypothesis that omega-3 fatty acids improve tolerance for adjuvant chemotherapy after biliary surgery with regard to increasing body weight and reducing cholangitis.

METHODS AND ANALYSIS

Study design

This study is a single-center, open-label, single-arm, historically controlled study of patients who are administered S-1 after surgical resection of BTC. The aim of this study is to examine any improvement in completion rates of adjuvant chemotherapy by the administration of omega-3 fatty acids. All participants are scheduled to receive administration of four cycles of S-1 adjuvant chemotherapy, and oral omega-3 fatty acids during chemotherapy (Figure 1). This study includes a 3-year period of registration and one year of follow-up. This study protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement.

Sample size

The sample size was calculated based on the primary endpoint. This study is planned for 55 participants. A previous study reported that the percentage completion for four courses of S-1 chemotherapy was 76% in patients with BTC [3]. The reasons for discontinuance were adverse events (12%) and early recurrence (12%). We set 0.76 as the threshold proportion. Aoyama et al. reported that administration of nutritional support with omega-3 fatty acids caused a 100% completion rate for adjuvant chemotherapy in gastric cancer [6]. We set 0.9 as the estimated proportion for our study because BTC has about a 10% early recurrence rate.

Under an estimation of 0.76 for a threshold proportion and 0.9 for an estimated proportion, 51 participants are required for a one-sided significance level 0.025 and a power

of 1–0.2. There is a potential for a few dropouts from follow-up, so we plan for a total of 55 participants. If the early recurrence rate is the same as that of a previous report (12%) and the estimated proportion is 0.88, the power will be 0.56.

There are about 25 target surgical operations in our institution per year, so we set a registration period of 3 years.

Study participants

Potential participants are all patients in whom the surgical resection of BTC is performed during the study period. After surgical resection, participants are recruited and must provide written, informed consent before enrollment for the study. Participants who satisfy all the inclusion criteria and none of the exclusion criteria (Table 1) are enrolled in this study.

Schedule of the study

Table 2 shows the schedule of the study. After written, informed consent, researchers perform a screening test and check inclusion and exclusion criteria. If participants meet all eligibility criteria, they are registered in the study. No protocol treatment before registration and no revocation after registration are permitted. After all data are entered and registration numbers given, then patients are counted as registered. Participants undergo protocol treatment within 14 days after registration.

After registration, this study consists of a maximum two-week pre-observation period, 24-week treatment period, and four-week post-observation period. During the pre-observation period, participants taking an antilipemic agent change their drug to omega-3 fatty acid formulations.

Participants do not receive any anti-cancer treatment after protocol treatment until the recurrence of cancer.

Intervention

S-1

Participants receive S-1 orally with the amount calculated considering body surface area and creatinine clearance (Table 3). Body weight for this calculation is that at registration. They receive S-1 twice daily for 28 consecutive days, and the drug is withdrawn for the next 14 days (one cycle). This administration of S-1 is repeated every six weeks for up to four cycles. If a cycle ends within 42 days for some reason, participants undergo an additional cycle until the end of the 24-week treatment period. A cycle is not newly started after a treatment period, and ongoing cycles on day 168 of the treatment period continue to the end.

Participants should meet the following criteria when starting the treatment from the second cycle: 1) Eastern Cooperative Oncology Group (ECOG) performance status is 0, 1, or 2; 2) neutrophil count is 1,000 cells/μL or higher; 3) platelet count is 5,0000 cells/μL or

higher; 4) aspartate aminotransferase (AST) concentration is less than three times the upper limit; 5) alanine aminotransferase (ALT) concentration is less than three times the upper limit; 6) total bilirubin (T-Bil) concentration is less than 1.5 times the upper limit; 7) percutaneous arterial oxygen saturation is 90% or more, and respiratory status dose is not worse than at registration; and 8) researchers consider the treatment can be performed safely.

Omega-3 fatty acid formulations

All participants take LOTRIGA 2g® (Takeda pharmaceutical Co. Ltd), which includes 2 g of omega-3 fatty acids from day one to day 168 of the treatment period.

Withdrawal and reduction of the drug

If any adverse events are observed, researchers should withdraw S-1 and omega-3 fatty acid formulations. When participants meet the criteria for restarting within seven days, they restart S-1 as the same cycle. In this case, the next cycle can start from the scheduled day with at least a seven-day interval. When participants do not meet the criteria for restart after more than 8 days, the cycle stops at that time. If participants meet the starting criteria, they can start a new cycle. When participants do not meet the restart criteria after more than 42 days since the last day of taking S-1, they should stop the protocol treatment.

When restarting S-1 after withdrawal due to an adverse event, participants get a reduced dose of S-1. The daily dose of S-1 is reduced from 120 mg to 100 mg, from 100 mg to 80 mg, from 80 mg to 60 mg, or from 60 mg to 50 mg. Participants have two opportunities

for reducing the drug. Participants taking 50 mg of S-1 should stop the protocol treatment.

If participants develop febrile neutropenia, they should be evaluated for infectious status, and antimicrobial agent or granulocyte colony stimulating factor (G-CSF) administered.

Adherence

Researchers provide patient compliance instructions, and confirm at each visit the remnant of drugs and whether participants have ingested the correct dose of S-1.

Concomitant drugs and therapy

There are no concomitant drugs and therapy during this study.

Outcomes

Primary outcome

The primary outcome is the completion rate of adjuvant chemotherapy. Completion is defined as participants who can finish a total of four cycles of S-1 adjuvant chemotherapy, regardless of relative performance index (RP: proportion of actual dose per expected dose) after surgical resection of BTC.

Secondary outcomes

Secondary outcomes are as follows:

1) Post-operative cholangitis

2) Time to recurrence or distant metastasis

3) Changes in nutritional index from registration until the completion of chemotherapy

4) Changes in the lymphocyte blast transformation test induced by phytohemagglutinin (PHA) and concanavalin A (Con-A) during adjuvant chemotherapy

5) Change in diamine oxidase serum activity (DAO) during adjuvant chemotherapy

6) All adverse events after medication. A modified version of The Common Terminology Criteria for Adverse Events (CTCAE v4.0) is used to grade adverse events.

Prospective adverse events are as follows: coagulation disorder, lipid metabolism disorder, abnormality of laboratory data, fatigue, fever, gastrointestinal disorders, skin and subcutaneous tissue disorders, metabolism and nutrition disorders, nervous system disorders, blood and lymphatic system disorders, eye disorders, infections and infestations, injury and procedural complications.

Data analysis

Participants who are recruited into this study and are administered one of the test drugs at least once are included in the full analysis set (FAS). Participants who cannot provide baseline data and have serious violations of protocol are not included in the FAS. The per protocol set (PPS) is defined as participants in the FAS who comply with exclusion criteria, concomitant drugs, and concomitant therapy. An analysis of FAS is performed for each

outcome. Safety is analyzed in PPS. All analysis is performed with fixed data.

Primary outcome

The point estimate of the completion rate of S-1 and 95% confidence interval (CI) is calculated. Participants who complete S-1 are defined as participants who have performed four scheduled cycles of S-1, or have continued treatment for 168 days.

Secondary outcomes

Analyses of secondary outcomes take the form of simple descriptive statistics (e.g., proportions and interquartile ranges (IQRs), means and standard deviations [SDs]) and where appropriate, point estimates of effect sizes (e.g., mean differences) and associated 95% CIs. Recurrence-free survival curves are calculated using the Kaplan–Meier method, and 95% CIs are calculated.

Safety analysis

In the evaluation of the safety of the study, all adverse events due to the study are counted. Severe adverse events are defined as the following three events: Grade 4 of a non-blood system disorder, early mortality within 30 days after the last treatment, or mortality causally related to the protocol treatment.

Interim analysis is to be performed in this study. All statistical analyses are conducted using JMP software (version 13.0, SAS, Cray, NC, USA).

Data management

Researchers make case report forms (CRF) for each participant. The modification of a CRF is permitted only when it does not exceed the prescribed range and is not a burden to participants. The principal investigator confirms and signs the CRF. CRFs are made at registration (within two weeks), during a treatment period (each two cycles), at the end of treatment (within 30 days), and in the follow-up period.

Data and safety monitoring

Independent data and safety monitoring is conducted. The following materials are reviewed every six months: informed consent obtained and signed, participant retention, study implementation system, study safety and data, and study progress.

Confidentiality

All study-related information is stored securely at the study site and identified by a coded number only to maintain participant confidentiality. All participant information is stored in locked file cabinets in areas with limited access. All records that contain names or other

personal identifiers are stored separately from study records identified by code number. All local databases are secured with password-protected access systems. A participant's study information is not be released outside of the study without the written permission of the participant.

ETHICS AND DISSEMINATION

This study is conducted according to the declaration of Helsinki, Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan, and Management Guidelines of Conflict of Interest in our institution. The protocol has been approved by the Institutional Review Board of Kobe University Hospital (No 290086).

Researchers explain the study to potential participants using information sheets. Patients are given the opportunity to ask questions and have enough time to consent to the study. Researchers obtain written consent from patients willing to participate in the study. Information sheets and consent forms are provided for all participants involved in the study.

The results of this study will be disseminated through academic conferences and peer-reviewed journals. All authors will review and approve the paper before publication. If there are several papers related to this study, the investigator will appoint each author.

DISCUSSION

The biliary tract is divided into four anatomical components: intrahepatic bile duct, extrahepatic bile duct, gallbladder, and ampulla of vater. Cancer staging is different for each origin, and the prognosis also differs. To select proper patients for this study, we decided that the indication criteria was Stage Ⅰ or higher for intrahepatic cholangiocarcinoma and Stage Ⅱ or higher for other BTCs according to a Japan Clinical Oncology Group (JCOG) 1202 trial [14] which was conducted on the basis of the following data. The five-year survival rate in Stage I from a 2008 to 2013 nationwide survey in Japan [15] was 91% for gallbladder cancer and 92% for ampullary region cancer; a difference compared with other digestive organ cancers in Stage Ⅰ was not found. Although the five-year survival rate is somewhat low, being 74% for perihilar bile duct cancer and 78% for distal bile duct cancer, cancer deaths for these diseases is not common. Potential reasons for the low ratios include perioperative deaths and late complications such as cholangitis. Therefore, since we had regarded that these patients get few benefits from adjuvant chemotherapy, in principle adjuvant chemotherapy was not performed for Stage Ⅰ. In comparison, the 5-year survival rate of intrahepatic cholangiocarcinoma in patients with the tumor, less than 2 cm in diameter, that corresponds to Stage I is 36.3% in Japan's National Primary Liver Cancer Follow-up Survey (the 18th), which was worse than the same stage for other BTCs. Therefore adjuvant chemotherapy seems to be beneficial for patients of Stage I intrahepatic cholangiocarcinoma.

Though the target for adjuvant chemotherapy for BTC is still controversial, we have selected the relatively poor-prognosis group in BTC. Moreover, to align the patients post-surgical background, we have selected the type of surgical operation, which includes biliary reconstruction.

The most common reason for the interruption of adjuvant chemotherapy was an adverse event in BTC [3]. Seo et al. [16] reported that hypoalbuminemia (<3.5 mg/dL) was associated with adverse events greater than grade 3 in gastric cancer. Hypoalbuminemia is also a risk factor for the interruption of adjuvant chemotherapy in patients with pancreatic cancer[17]. The albumin level is one of the indications of nutritional status. According to a previous study, body weight loss is associated with the continuance of adjuvant chemotherapy [6], and nutritional intervention, including omega-3 fatty acids, has an effect on several cancers [7] [8].

It is well known that chemotherapy including 5-fluorouracil (5-FU) causes gastrointestinal injury [18]. Five-fluorouracil also showed toxicity to helper T cells in mice, which seemed to cause immunosuppression [19]. These changes may result in BT [20]. From the viewpoint of immunity and infection, omega-3 fatty acids play a preventive role. Matsunaga et al. reported that a diet containing omega-3 fatty acid suppressed the thickening of mucosa, submucosa, and the muscular layer due to inflammation in mice compared with a control diet [21]. Moreover, omega-3 fatty acids inhibit infiltration of inflammatory cells into the muscularis

mucosa[19]. In addition, the rate of BT and ileal change in pre- and postoperative feeding with omega-3 fatty acids in rats with a common bile duct ligation were the same as that of the control group, while BT and atrophy of intestinal mucosa in the postoperative feeding group increased compared to the control group [22]. From these reports, it is thought that the administration of omega-3 fatty acids from the start of chemotherapy, when intestinal mucosal disorder is not yet occurring, can keep the intestinal mucosa normal and prevent intestinal inflammation and the interruption of anticancer agents by infection.

In addition to the nutritional aspect, omega-3 fatty acids have an anti-inflammatory effect. After biliary reconstruction, it is easy for patients to experience cholangitis and stop chemotherapy. Omega-3 fatty acids are known to calm acute inflammation by regulating inflammatory cytokines [9]. Furthermore, *in vivo* experiments showed suppression of the proliferation of cancer cells by inducing apoptosis in several cancers [10 12 13].

In this study, we expect that omega-3 fatty acids will improve the completion rate of adjuvant chemotherapy and lead to a good prognosis in patients with BTC. However, this study may not be generalizable to other chemotherapy regimens since only S-1 is the target as adjuvant chemotherapy.

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Registry This study is registered at UMIN Clinical Trials Registry (UMIN000031247) and Japan Registry of Clinical Trials (jRCTs051180007).

Registration Data Set: https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000035677

Registration Data Set: https://jrct.niph.go.jp/detail/349

Contributors KU designed the study, and wrote the initial draft of the manuscript. TA and TF contributed to analysis and interpretation of data, and assisted in the preparation of the manuscript. DT, MA and YH contributed to analysis and interpretation of data. MA, HM, SK, KK, ST, MT, HT and MK contributed to collect the clinical data. All authors approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding This study has received no specific grant from any funding agency in the public, commercial or not-for- profit sectors.

Competing interests None declared.

Patient consent Obtained

Patient and Public Involvement We did not involve patients or the public in our work

Ethics approval Kobe University Clinical Research Ethical Committee

Issue date: September 20, 2018

Protocol amendment number: 1.4

Table 1 Inclusion and exclusion criteria for patients

Inclusion criteria
1) Pathologically diagnosed as adenocarcinoma (in case of a combined cancer, adenocarcinoma is dominant) 2) UICC stage is as follows: Stage Ⅰ or higher in intrahepatic cholangiocarcinoma Stage Ⅱ or higher in perihilar bile duct cancer, distal bile duct cancer, gallbladder cancer and ampulla region cancer 3) age 20 – 80 years or younger 4) ECOG performance status is 0 or 1 5) CT and MRI show no metastases, and moderate or less pleural effusion* or ascites** 6) Performed pancreaticoduodenectomy, hepatectomy, bile duct resection, caudal lobectomy and/or cholecystectomy, with D1 or more extensive lymphadenectomy*** 7) No history of any prior chemotherapy or radiation therapy 8) 14 to 70 days from surgical resection 9) Good oral intake 10) No watery diarrhea Meets all the following laboratory data values within 14 days before registration a) neutrophils $\geq$ 1,200/mm^3 b) platelets $\geq$ 10,000/mm^3 c) hemoglobin $\geq$ 8.0g/L d) total bilirubin $\leq$ 2.0 mg/dL e) AST $\leq$100 IU/L f) ALT $\leq$ 100 IU/L g) serum creatinine $\leq$ 1.2 mg/dL h) creatinine clearance (CC) $\geq$ 40 mL/min (CC calculated by Cockcroft–Gault Equation) 11) Written informed consent
Exclusion criteria
1) Allergic predisposition or drug hypersensitivity 2) Double or multiple cancers diagnosed or treated in the past five years. (except for carcinoma *in situ*) 3) Active infectious disease (except for virus hepatitis) 4) Fever above 38ºC

5) Current pregnancy, breastfeeding, planning to become pregnant during the study
6) Psychological disease and/or symptoms
7) Severe liver or renal dysfunction
8) Uncontrollable hypertension or diabetes mellitus
9) Uncontrollable ischemic heart disease:
   - developing angina or worsening within three weeks
   - developing myocardial infarction within six months
10) Taking flucytosine, phenytoin, or warfarin potassium
11) Interstitial pneumonitis, pulmonary fibrosis, and pulmonary emphysema
12) Taking omega-3 fatty acid formulations
13) Considered unsuitable by their attending physician

CT, computed tomography; MRI, magnetic resonance imaging; UICC, Union for International Cancer Control; ECOG, Eastern Cooperative Oncology Group; AST, aspartate aminotransferase; ALT, alanine aminotransferase

*Moderate plural effusion is defined as effusion occupying one third of the lung field by chest X-ray. **Moderate ascites is defined as ascites within the pelvic cavity. ***In the case of intrahepatic cholangiocarcinoma without invasion to the hepatic portal region, lymphadenectomy is not necessary.

Table 2 Schedule of enrollment, interventions, and assessments.

	STUDY PERIOD							
	pre-observation	**treatment period**						**post-observation**
TIMEPOINT (week)	*14 days before registration*	*0*	*2, 4, 6*	*9*	*12*	*15 ,18, 21*	*Close-out, 24*	*28*
VISIT	1	2	3, 4, 5	6	7	8, 9, 10	11	12
ENROLLMENT:								
Eligibility screen	X							
Informed consent	X							
Background Performance status Blood test	X							
INTERVENTIONS:								
S-1		←	—	—	—	—	→	
LOTRIGA		←	—	—	—	—	→	
ASSESSMENTS:								
Symptoms			X	X	X	X	X	X
Body weight			X	X	X	X	X	X
General status					X		X	X
Blood test			X	X	X	X	X	X
ConA, PHA, DAO	X				X		X	
Adverse event			X	X	X	X	X	X

Background includes age, gender, body weight, height, past history, complication, date of diagnosis, family history, medication, and operative information (e.g., date, method, blood loss,

operation time). Screening tests are general status, blood test, urinary test, computed tomography and electrocardiogram. General status includes blood pressure, body temperature, pulse rate and respiratory rate. Blood test includes complete blood count, differential count of leukocytes, total protein, albumin, total bilirubin, creatinine, aspartate transaminase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, Na, K, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride. ConA, concanavalin A; PHA, phytohemagglutinin; DAO, diamine oxidase

Table 3 Dose of S-1

	Dose of S-1	
Body surface area (m^2)	50 mL/min ≤ Ccr < 60 mL/min	60 mL/min ≤ Ccr
under 1.25	60 mg/day	80 mg/day
1.25 to 1.5	80 mg/day	100 mg/day
over 1.5	100 mg/day	120 mg/day

Ccr, creatinine clearance

Figure legend

Figure 1 Summary of study.

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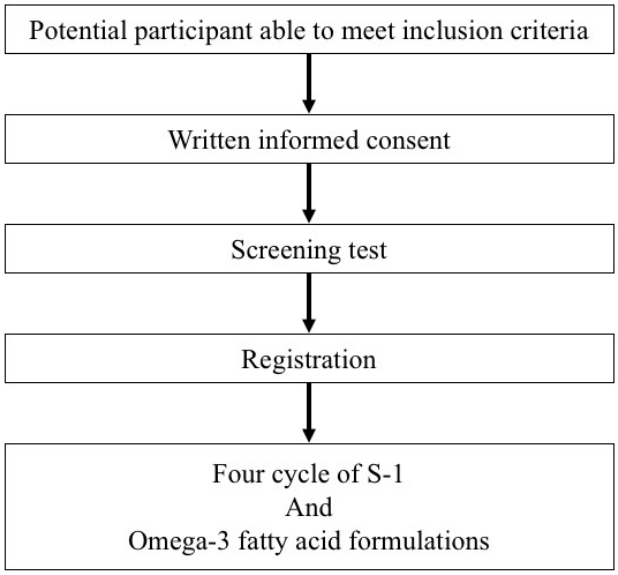

Figure1. Summary of study

Summary of study

190x275mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	18
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	18
Protocol version	#3	Date and version identifier	18
Funding	#4	Sources and types of financial, material, and other support	18
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	18
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	N/A

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13-14
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	5-6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6-7
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8-9
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	9-10

Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-12
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA this study is single-arm
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA this study is single-arm
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA this study is single-arm

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA this study is single-arm
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA this study is single-arm
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13-14
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12-13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12-13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found,	14

		if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A not perform interim analysis
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	14
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	14
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A

Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	15
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A only in japanese
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A