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Phase I Safety and Clinical Activity Study of Thymoquinone in Patients with Advanced Refractory Malignant Disease.

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Abstract:

Purpose: This phase I study was conducted to determine the general toxicities of thymoquinone in humans, as well as any anti-cancer effects that the drug may have in patients with advanced cancer for which there were no standard curative or palliative measures.
Patients and methods: Adult patients with solid tumors or hematological malignancies who had failed or relapsed from standard therapy were included in this study.
Patients who were at least 18 years of age with an Eastern cooperative oncology group performance status (ECOG) of ≤ 2 received thymoquinone orally at a starting dose level of 3, 7, or 10mg/kg/day. Dose escalation proceeded according to a modified Fibonacci design.
Results: All 21 patients received at least one week of treatment, with a median of 3.71 weeks (range 1 week to 20 weeks). No side effects were reported and the maximum tolerated dose (MDT) was not identified. No anti-cancer effects were observed.
Conclusion: On the basis of this study, thymoquinone was well tolerated at a dose ranging from 75mg/day to 2600mg/day. Neither toxicities nor therapeutic responses were reported.

Keywords: Thymoquinone, Cancer, Toxicity.

Introduction:

Thymoquinone is an important constituent of the Nigella sativa plant (Black seed). The plant has been used in herbal medicine for more than 2000 years, and belongs to the Ranunculaceae family of flowering plants.[1] Both N. sativa and its ingredient thymoquinone are reported to possess numerous beneficial pharmacological effects,[2, 3] however, thymoquinone and dithymoquinone have also been found to be cytotoxic in several types of parental and multi-drug resistant human tumor cell lines.[4 -6] Therefore, we performed this phase I dose escalating study in order to evaluate the maximum tolerating dose of thymoquinone, in addition to its preliminary anti-tumor activity in patients with advanced malignant diseases.

Methods:

Eligibility criteria

Patients ≥ 18 years of age, with histologically confirmed advanced malignant disease for which there were no standard curative or palliative measures were included in this study. Three patients declined the standard therapeutic option of chemotherapy. Eligible patients were required to have an Eastern oncology cooperative group (ECOG) performance status score of ≤ 2. The patients were required to have an adequate liver function test and renal function test (Bilirubin ≤ 1.5 X upper limit of normal [ULN], ALT and AST ≤ 3X ULN, Creatinine 1.5 X ULN, and BUN 2 X ULN). The complete blood count requirement included: an absolute granulocyte count of ≥ 1500/µl, platelet ≥ 100,000 / µl and hemoglobin ≥ 9 gm/dl. Patients were excluded if they had brain metastasis, had suffered from a myocardial infarction in the last 4 months, had a congestive heart failure functional status ≥ II, or had coagulopathy disorder. The research and ethical committees of our university approved the protocol. After explaining the possible side effects and outcomes of thymoquinone, written consent was obtained from each patient who participated in the study.

Drug and dose escalation

Thymoquinone was obtained from Frinton laboratories, USA, as a yellow crystalline powder. The drug was then prepared as 10 mg, 20mg, 100mg, 200mg, and 400mg capsules at King Faisal University laboratory-Dammam.

This phase I trial was an open-label, non-randomized, dose-finding study of oral thymoquinone. The initial dose was chosen based on the LD50 thymoquinone toxicity of 10mg/kg in rats treated intraperitonealy with thymoquinone.[8] However, the LD50 for acute oral administration of thymoquinone in mice was reported to be 2.4 g/kg.[9]

It was planned that cohorts of at least 3 patients were to be evaluated at each dose level. The starting dose was 10% of LD50 in animals, at 10mg/kg/day. The first cohort of patients was given 1mg/kg/day, the second cohort was given 6mg/kg/day, and the third cohort was given 10mg/kg/day. The dose was escalated in each cohort according to the modified Fibonacci series.[10, 11] Thymoquinone was increased by 100%, 66%,

50%, and then 33% on a weekly basis. The drug was discontinued if toxicities were reported or if disease progression was detected.

Pre-treatment and follow up evaluation

At the start of therapy, a history, physical examination, and a histological confirmation of the malignant tumor was performed. In addition, blood samples were obtained to examine changes in CBC, RFT, LFT, lipid profiles, RBS, ESR, tumor markers (CEA, CA125, CA199, CA153, BHCG, AFP, PSA, LDH), PT and PTT. These blood tests were also performed weekly during the course of therapy, and any changes to weight, vital signs, and symptom presentation were recorded during a general physical examination. Imaging, including CAT scans and ultrasounds were performed at the start of therapy and throughout the course of the study to determine of disease progression had occurred.

Results:

Patients

Twenty-one patients enrolled to receive thymoquinone treatments from November 2006 to September 2007 (Table 1). Ten patients dropped out or declined to continue treatment in the first two weeks of therapy. These patients elected to seek medical advice in another hospital or for other personal reasons. In 11 patients, thymoquinone was discontinued due to clinical or laboratory evidence of disease progression.

Drug safety

Safety assessments were performed weekly. During the treatment period, no adverse symptoms related to thymoquinone were reported. In addition, laboratory tests: (CBC, RFT, LFT, RBS, Lipid profile, ESR) were not significantly different from baseline values.

Tumor response

In the first 2 weeks of treatment, four patients demonstrated a modest weight gain of 2 kg, with an overall improvement in their general condition. In addition, tumor markers decreased in these patients however, the decrease was not by more than 25% of baseline levels. Therefore, no anti-tumor effects were confirmed by the tumor markers or imaging.

Discussion:

In this first human trial, patients with advanced malignant cancers who were treated with thymoquinone were able to tolerate the drug at oral doses up to 2600 mg/day. The maximum dose tolerated was not reached in this study. No clinical toxicities or laboratory abnormalities were reported. Because there was no discontinuation of treatment as a result of side effects from thymoquinone, all patients were kept on the drug for the duration of the study unless there was evidence that their malignant diseases had progressed. One patient remained on thymoquinone for 20 weeks with a dose of 1000mg/day and another patient had 2600mg/day for 1 week. Neither of these two patients had any side effects. The absence of side effects in humans is in

agreement with the extremely low toxicities of oral thymoquinone administration in mice, which was reported to be 2.4gm/kg.[9]

Table1. Patient's characteristics.

Characteristics	No. of patients	%
Age, Years		
Median 56		
Range 23-92		
Sex		
Male	11	52
Female	10	48
ECOG PS		
0	3	14
1	10	48
2	8	38
Tumor site		
Colonic adenocarcinoma	3	14
NSCL	3	14
Breast cancer	3	14
CUO	2	9.5
RCC	2	9.5
HCC	2	9.5
Others	6	29

Table2. Patients recruited with the duration and dose of thymoquinone.

Serial numbers of patients	Diagnosis	Duration on Thymoquinone (Weeks)	Maximum dose of Thymoquinone (mg/day)
1	NSCLC	5	400
2	Prostatic carcinoma	1	75
3	NSCLC	5	500
4	HCC	3	250
5	Diffuse large B- cells Lymphoma	2	100
6	Colonic adenocarcinoma	2	100
7	Colonic adenocarcinoma	6	750
8	CUO	2	150
9	Breast carcinoma	7	800
10	Pancreatic carcinoma	1	85
11	Colonic adenocarcinoma	20	1000
12	Gastric carcinoma	5	600
13	Liomyosarcoma	3	500
14	Gastric Carcinoma	3	1200
15	CUO	4	2000
16	Pancercotic adenocarcinoma	1	500
17	Renal cell carcinoma	3	400
18	HCC	1	400
19	Breast carcinoma	2	400
20	RCC	1	800
21	NSCLC	3	2600

NSCLC, Non small cell lung carcinoma; HCC, Hepatocellular carcinoma; CUO, Carcinoma of Unknown Origin.

Because this was a phase I study, toxicity was the primary end point. However, in the first two weeks of therapy, there was an improvement in the general condition of some patients. Four patients saw an increase in their body weight and a reduction of the number tumor markers, though this reduction was not more than 25% from baseline. Imaging studies identified no significant objective response.

Although this study has several limitations including the fact that the MTD was not identified and the blood level of thymoquinone was not measured, the results indicate a wide margin of safety for its use in humans. The starting dose of thymoquinone needs to be increased to reach the MTD in order to discover a possible role for thymoquinone in the treatment of patients with advanced malignant diseases.

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