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Original Article

Safety, tolerability, and pharmacokinetics of mitoxantrone hydrochloride injection for tracing in patients with gastric cancer: a single-blind, single-center, phase I clinical trial

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Abstract: Mitoxantrone Hydrochloride Injection for Tracing (MHI), a modified new drug marketed in China, has been approved by the National Medical Products Administration for lymph node tracing in thyroid cancer and sentinel lymph node biopsy in breast cancer. This single-center, single-blind, dose-escalation phase I clinical trial aimed to investigate the safety of MHI on lymph node tracing in gastric cancer. In this study, four dose groups (1.0 mL, 1.5 mL, 2.0 mL, and 3.0 mL) with 3 gastric cancer patients in each group were set. The safety, tolerability, pharmacokinetics and preliminary efficacy of different doses were investigated. Results showed that none of the patients experienced dose-limiting toxicity or developed serious adverse events or adverse drug reactions. Pharmacokinetic analyses revealed minimal absorption of the tracer, resulting in low and transient blood drug concentrations across all participants. The mean time to peak concentration was (0.561 ± 0.3728) h (with mean peak concentration (C_{max}) of 10.300 ng/mL), (0.500 ± 0.0167) h (mean C_{max} of 13.687 ng/mL), (0.494 ± 0.0096) h (mean C_{max} of 30.933 ng/mL), and (0.661 ± 0.2791) h (mean C_{max} of 21.067 ng/mL) in the 1.0 mL, 1.5 mL, 2.0 mL, and 3.0 mL dose groups, respectively. The mean lymph node staining rates were 21.0%, 24.7%, 32.5%, and 44.5%, and the mean metastatic lymph node staining rates were 20.6%, 36.1%, 42.4%, and 21.0% in each group. This study confirmed that MHI was safe, well-tolerated, and had low systemic effects when used for lymphatic tracing of gastric cancer, and the tracing effect was better in the 3 mL dose group. This trail was registered on the website of Centre for Drug Evaluation State Drug and Food Administration (http://www.chinadrugtrials.org.cn/index.html) with the name of clinical study of lymphatic tracer in lymph node tracing of gastric cancer, the code was CTR20201906.

Keywords: Mitoxantrone hydrochloride injection for tracing, gastric cancer, lymph nodes, pharmacokinetic, safety

Introduction

Gastric cancer is one of the most common cancers, with more than 1 million new patients and 769,000 deaths worldwide by 2020 [1, 2]. It is particularly prevalent in East Asia, Eastern Europe, Central America, and South America. The latest statistics from the National Cancer Center in China show that gastric cancer ranks third in both morbidity and mortality [3]. In China, more than 70% of gastric cancer patients are already in the middle and late stage when diagnosed and have lymph node metastasis [4, 5]. High surgical quality, thorough lymph node dissection, and accurate pathological staging are the keys to achieve radical treatment and improve survival [6, 7].

How to avoid inadequate lymph node dissection or blindly expanding the dissection area becomes a challenge for surgeons. In recent years, lymphatic navigation techniques have been widely developed. These techniques involve injecting a lymphatic tracer around the tumor, which then migrates through the lymphatic system into the lymph nodes, allowing lymph node imaging [8]. Surgeons can improve the quality of lymph node dissection and make

accurate lymph node staging [9, 10]. Different types of lymphatic navigation techniques have brought different clinical results for minimally invasive gastric cancer surgery [11, 12]. At present, lymphatic tracing methods mainly include blue dyes, carbon nanoparticles, radionuclide tracing method, fluorescence imaging technique [13]. Methylene blue, as a substitute for isosulfan blue and patent blue in China, does not bind to plasma proteins due to the lack of sulfonic acid groups. Therefore, its lymph node specificity has been revealed to be inferior [14]. Carbon nanoparticles, in suspension form, present challenges in dose control, thus affecting the trace effect [15]. Radionuclide tracing method is difficult to promote due to the drawbacks such as expensive equipment, time-consuming, complex operation process, and the radioactive nuclide material [16]. Similarly, fluorescence detection method is also expensive with relatively complex operation process, limiting their general application [17]. Therefore, there is an urgent need for a new lymphatic tracer that is readily accessible to patients and user-friendly, with verified efficacy and safety in the field of gastric cancer.

Mitoxantrone hydrochloride injection for tracing (MHI) demonstrates exceptional lymph node targeting capabilities. After injection around the tumor, mitoxantrone self-assembles locally to form nanocrystals with a particle size of approximately 100 nm. These nanocrystals become enriched in lymph nodes after passing through the interstitial space into lymphatic capillaries and stain lymph nodes blue, thus making them more detectable [18]. MHI holds promise as an ideal lymphatic tracer in gastric cancer surgery because of its easy operating and good visibility. The aim of this study was to evaluate the safety and tolerability of MHI used as lymphatic tracer in patients with gastric cancer and to determine the optimal dose range, which could provide a rational basis for the dose selection and evaluation criteria for subsequent phase II and III validation clinical trials with large sample.

Materials and methods

Study design

This is a single-blind, single-center, dose-escalation phase I clinical trial with human tolerability and pharmacokinetic testing. The dose escalation protocol was based on a traditional 3+3 design. Four dose groups of 1.0 ml, 1.5 ml, 2.0 ml and 3.0 ml with 3-6 cases in each group were set. The drug concentration was 5 mg/ml. Two or more dose groups could not be conducted simultaneously. The trial started with the 1.0 ml group and first enrolled 3 subjects. Once a DLT event occurred in this group, 3 additional subjects were to be enrolled. If 2 or more subjects in this group experienced a DLT during the observation period, the dose escalation study was terminated. The next dose group (2.0 ml) was administrated if no DLT was observed within 14 ± 2 days. Each group was gradually carried out according to the above rules. The trial may be terminated if the injection site is saturated with the drug injection dose carrying. If saturation does not occur at the completion of the 4 groups and safety is confirmed, the principal investigator may determine whether an additional 4.0 ml dose group is needed for observation. The safety evaluation of each group scheduled through return visits at 4 ± 1 days, 14 ± 2 days and 28 ± 3 days after dosing.

Participants

Patients who were proposed to undergo radical gastric cancer surgery at the Fourth Hospital of Hebei Medical University from September 2020 to December 2021 were prospectively included. Inclusion criteria: (1) patients who voluntarily signed the informed consent form; (2) patients with an age of 18-75; (3) patients with gastric adenocarcinoma, cN+, T2-T4, M0; (4) patients who were proposed for radical gastric cancer surgery; (5) patients with no clear contraindications to surgery seen in routine preoperative examination; (6) patients with good compliance. Exclusion criteria: (1) patients who had received previous chemotherapy or radiotherapy; (2) patients with recurrent gastric cancer; (3) patients allergic to anthraquinones; (4) patients with metastases to the peritoneum, liver and other organs; (5) patients with other concomitant malignant tumors; (6) patients with hemoglobin < 90 g/L, or absolute neutrophil count < 1.5×10^9/L, or platelet count < 75×10^9/L; (7) patients with alanine aminotransferase or aspartate aminotransferase > 1.5 times upper the limit of normal value; (8)

patients with serum creatinine > 1.5 times upper the limit of normal value; (9) patients with mental illness, alcohol dependence, or sedative drug dependence; (10) pregnant or lactating or planning to become pregnant within 6 months; (11) patients who were participating in another clinical trial or had participated within the last 3 months. This study was approved by the ethics committee of the Fourth Hospital of Hebei Medical University and registered on the website of Centre for Drug Evaluation State Drug and Food Administration (code: CTR20201906, http://www.chinadrugtrials.org.cn/index.html). All patients provided informed consent.

Surgical procedures

Patients with gastric cancer underwent laparoscopic exploration and abdominal exfoliative cytology after general anesthesia. Dissection was performed after excluding peritoneal metastases and the presence of free cancer cells in the abdominal cavity. After complete exposure of tumor site, MHI (5 mg/ml, provided by Shenzhen China Resources Jiuchuang Pharmaceutical Co., Ltd.) was injected into the subserous membrane of the stomach at a distance of approximately 1.0 cm from the edge of the tumor lesion at multiple points. Dissection surgery was performed after lymph nodes were visualized.

Evaluation endpoint

(1) Dose-Limiting Toxicity (DLT): According to the evaluation criteria of CTCAE 5.0 (Common Terminology Criteria for Adverse Events, Version 5.0), DLT is defined as Grade 3 or above drug toxicity in blood and skin, which affects daily life and requires treatment and discontinuation of the investigational drug. The occurrence of DLT was observed up to 14 ± 2 days after administration.

(2) Efficacy indicators: The number of lymph nodes detected per subject, the number of lymph nodes detected at station 1 and station 2. The staining rate of total lymph nodes and metastatic lymph nodes, the staining rate of metastatic lymph nodes at station 1 and station 2.

(3) Pharmacokinetics: Blood samples were collected for drug concentration testing at within 60 minutes prior to dosing and (15 ± 1) minutes, (30 ± 1) minutes, (60 ± 2) minutes, (120 ± 2) minutes, (240 ± 2) minutes, (360 ± 2) minutes after administration. All subjects who have received the test drug and completed the collection of biological samples at all-time points according to the protocol would be included in the pharmacokinetic study evaluation. Drug concentrations in blood were analyzed by LC-MS/MS at Shenyang Pharmaceutical University.

(4) Safety evaluation: All subjects treated with MHI were included in the safety evaluation. The safety evaluation was conducted until (28 ± 3) days after the end of the procedure, and was based on the comparison of the examination results within 28 days before screening and the results of postoperative laboratory examination, as well as the evaluation of adverse events based on the CTCAE 5.0 evaluation criteria. Safety evaluation includes the severity of adverse events, the study drug-related adverse events, the maximum tolerated dose and the safe dose range of the drug.

Statistical analysis

Continuous variables were summarized using descriptive statistics such as mean, standard deviation (SD), median, minimum and maximum values. Categorical variables were reported as frequency (including the number of missing values) and percentage of subjects in the corresponding classification. Data for individual subjects were provided in the data list by subject. The data list included all data collected for all recruited subjects from the beginning of the screening visit until the end of the study assessment. Subjects who failed screening were listed in a separate list.

Pharmacokinetic (PK) parameters were calculated by WinNolin software (version 8.0 or above), and demographic and safety data were analyzed by SAS (version 9.4 or above) software.

Analysis sets

Screening set: all subjects who signed the informed consent form. Full analysis set: all subjects who were successfully enrolled and received the study drug with at least one efficacy index. PK analysis concentration set: all

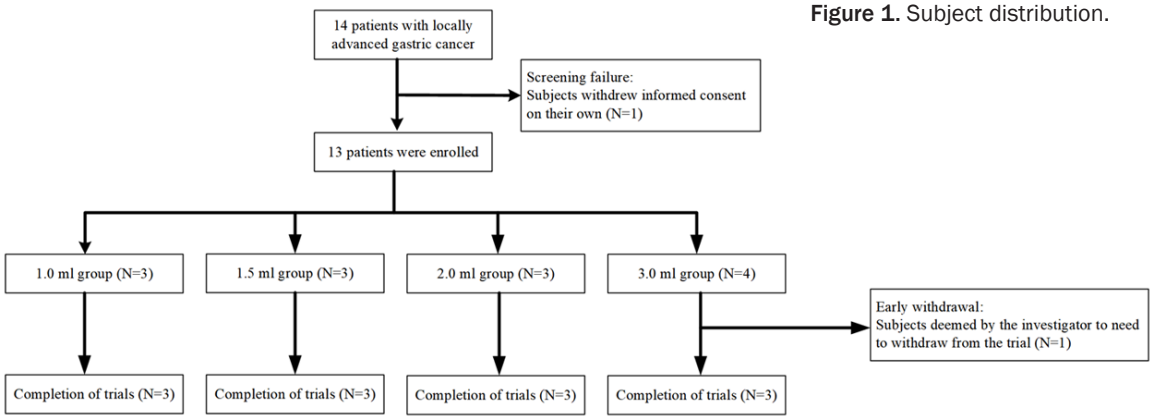

Figure 1. Subject distribution.

Table 1. Patient characteristics in full analysis set

	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=3)
Age (Median (range)), years	57.0 (53, 64)	58.0 (55, 69)	62.0 (62, 64)	66.0 (56, 71)
Gender, N (%)				
Male	3 (100.0)	1 (33.3)	3 (100.0)	3 (100.0)
Female	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)
Pathology, N (%)				
Adenocarcinoma	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
T stage, N (%)				
T2	0	0	0	1 (33.3)
T3	1 (33.3)	0	0	0
T4	2 (66.7)	3 (100.0)	3 (100.0)	2 (66.7)
N stage, N (%)				
N1	2 (66.7)	2 (66.7)	3 (100.0)	2 (66.7)
N2	1 (33.3)	1 (33.3)	0	1 (33.3)
M stage, N (%)				
M0	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)

N: number.

subjects enrolled and received the study drug with at least one blood concentration data during the trial. PK analysis parameter set: all subjects enrolled and who received at least one study drug with at least one validated PK parameter data. Safety analysis set: all subjects enrolled and who received the study drug.

Results

Demographics and baseline characteristics

A total of 14 subjects entered the trial screening in this study, with screening failure in subject number 01012 (**Figure 1**). A total of 13 subjects were enrolled, 12 of whom completed the trial and were included in the full analysis set, safety set, pharmacokinetics analysis concentration set and pharmacokinetics analysis parameter sets (Supplementary Table 1), and subject 01013 who withdrew prematurely without receiving dosing was not included in the analysis. A total of 18 sub-protocol deviations were recorded in 10 subjects in the full analysis population of the phase I clinical study, all with minor deviations (Supplementary Tables 2 and 3). Patients' baseline characteristics were listed in **Table 1**.

Pharmacokinetic analysis

The blood concentration data are shown in **Table 2**. Drug concentrations in blood were very low, with each subject having up to 4 concentration data that exceeded the Below the Quantization Limit (BQL). The highest concentration was only 37.4 ng/ml.

Table 2. Blood concentration analysis in pharmacokinetics analysis concentration set

Blood concentration	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=3)
Within 60 minutes before administration				
Above BQL, N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mean (SD)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Median (Min, Max)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)
15 ± 1 minutes after administration				
Above BQL, N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mean (SD)	6.357 (5.5451)	11.880 (9.5129)	25.700 (10.0802)	15.367 (1.6503)
Median (Min, Max)	8.870 (0.00, 10.20)	7.450 (5.39, 22.80)	26.600 (15.20, 35.30)	14.900 (14.00, 17.20)
30 ± 1 minutes after administration				
Above BQL, N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mean (SD)	8.063 (2.7931)	13.687 (10.7772)	30.933 (7.7106)	19.700 (3.1796)
Median (Min, Max)	8.520 (5.07, 10.60)	9.090 (5.97, 26.00)	33.000 (22.40, 37.40)	20.200 (16.30, 22.60)
60 ± 2 minutes after administration				
Above BQL, N (%)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)
Mean (SD)	5.760 (5.1976)	9.983 (10.6537)	14.713 (7.2888)	17.307 (7.3081)
Median (Min, Max)	7.180 (0.00, 10.10)	8.750 (0.00, 21.20)	14.300 (7.64, 22.20)	17.900 (9.72, 24.30)
120 ± 2 minutes after administration				
Above BQL, N (%)	3 (100.0)	1 (33.3)	3 (100.0)	2 (66.7)
Mean (SD)	0 (0.0)	4.500 (3.8979)	0 (0.0)	3.500 (6.0622)
Median (Min, Max)	0.000 (0.00, 0.00)	6.670 (0.00, 6.83)	0.000 (0.00, 0.00)	0.000 (0.00, 10.50)
240 ± 2 minutes after administration				
Above BQL, N (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
Mean (SD)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Median (Min, Max)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)
360 ± 2 minutes after administration				
Above BQL, N (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
Mean (SD)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Median (Min, Max)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)	0.000 (0.00, 0.00)

N: number; BQL: below the quantization limit; SD: standard deviation; Min: Minimum; Max: Maximum.

The mean blood concentration - time curve and mean blood concentration - time semi-logarithmic curve were plotted using pharmacokinetics analysis concentration set (**Figures 2**, **3**).

The pharmacokinetic parameters of each subject were calculated from a non-compartmental model using pharmacokinetics analysis parameter set, including maximum concentration (C_{max}), time to maximum concentration (T_{max}), area under the plasma concentration-time curve (AUC_{0-t}), elimination half-life ($t_{1/2}$), and elimination rate constant (λ_z). T_{max} in four groups (1.0 ml, 1.5 ml, 2.0 ml and 3.0 ml) were (0.561 ± 0.3728) h, (0.500 ± 0.0167) h, (0.494 ± 0.0096) h and (0.661 ± 0.2791) h, respectively. C_{max} in the above four groups were (10.300 ± 0.2646) ng/mL, (13.687 ± 10.7772) ng/mL, (30.933 ± 7.7106) ng/mL and (21.067 ± 4.2147) ng/mL, respectively. AUC_{0-t} in each group were (5.045 ± 2.1371) h*ng/mL, (17.202 ± 16.2601) h*ng/mL, (21.604 ± 6.7939) h*ng/mL, and (21.226 ± 11.6633) h*ng/mL, respectively (**Table 3**). Area under the plasma concentration-time curve (AUC) increased progressively with increasing dose of the drug. When the dose was increased to 3.0 ml, AUC did not increase significantly.

Lymph nodes detected and stained

The mean number and staining rate of lymph nodes were 48.0 ± 29.14 (21.0%), 46.7 ± 15.37 (24.7%), 60.3 ± 14.57 (32.5%), and 49.0 ± 8.54 (44.5%) in the 1.0 mL, 1.5 mL, 2.0 mL, and 3.0 mL group, respectively. Subgroup

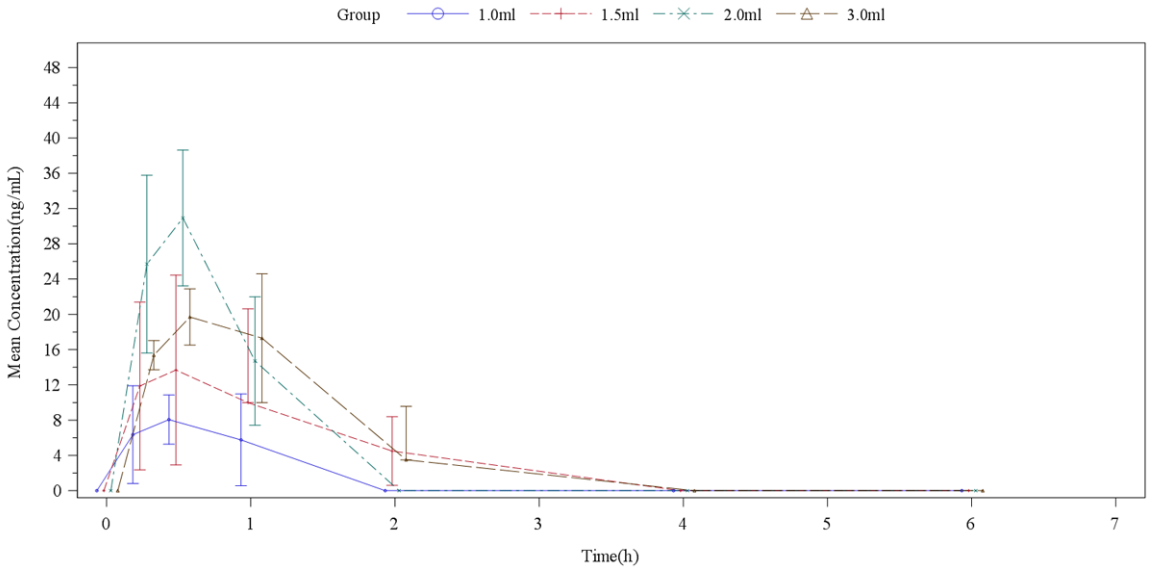

Figure 2. Mean blood concentration-time curve in pharmacokinetics analysis concentration set.

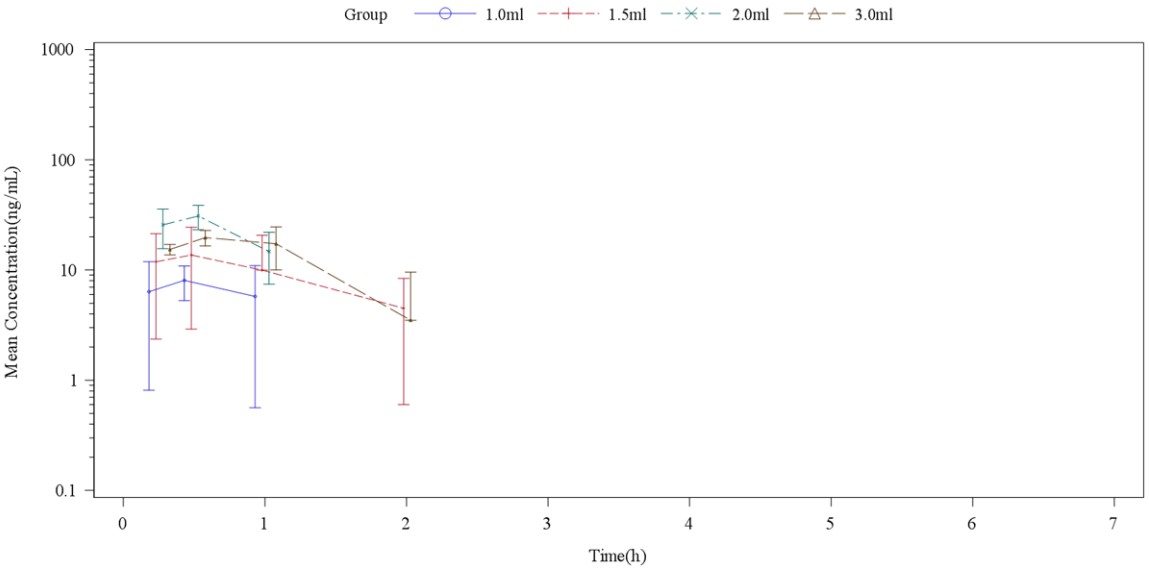

Figure 3. Mean blood drug concentration-time semi-logarithmic curve in pharmacokinetics analysis concentration set.

analysis based on the different stations of lymph nodes revealed that the mean number of lymph nodes detected at station 1 in the 1 ml group was 42.3 ± 24.58, while the remaining groups were 44.3 ± 16.07, 57.3 ± 13.58, and 44.3 ± 14.84, respectively. And for the station 2 lymph nodes, the number of detections in the 4 dose groups were 5.7 ± 5.13, 2.3 ± 1.15, 3.0 ± 1.00, and 4.7 ± 6.43, respectively (**Table 4**).

The mean number and staining rate of metastatic lymph nodes in 4 dose groups were 8.3 (20.6%), 3 (36.1%), 9.3 (42.4%) and 8.3 (21.0%), respectively (**Table 5**).

Safety analysis

No subjects withdrew from the trial due to adverse events. A total of 13 adverse events were recorded in 4 subjects in the safety analy-

Table 3. Pharmacokinetic summary

Pharmacokinetic parameters	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=3)
C_{max} (ng/mL)				
Number of cases (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
Mean (SD)	10.300 (0.2646)	13.687 (10.7772)	30.933 (7.7106)	21.067 (4.2147)
Median (Min, Max)	10.200 (10.10, 10.60)	9.090 (5.97, 26.00)	33.000 (22.40, 37.40)	22.600 (16.30, 24.30)
T_{max} (h)				
Number of cases (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
Mean (SD)	0.561 (0.3728)	0.500 (0.0167)	0.494 (0.0096)	0.661 (0.2791)
Median (Min, Max)	0.483 (0.23, 0.97)	0.500 (0.48, 0.52)	0.500 (0.48, 0.50)	0.500 (0.50, 0.98)
AUC_{0-t} (h*ng/mL)				
Number of cases (%)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
Mean (SD)	5.045 (2.1371)	17.202 (16.2601)	21.604 (6.7939)	21.226 (11.6633)
Median (Min, Max)	4.300 (3.38, 7.45)	15.187 (2.04, 34.38)	24.390 (13.86, 26.56)	16.724 (12.48, 34.47)
$t_{1/2}$ (h)				
Number of cases (%)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)
Mean (SD)	1.540	2.108 (1.9516)		
Median (Min, Max)	1.540 (1.54, 1.54)	2.108 (0.73, 3.49)		
λ_z (1/h)				
Number of cases (%)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)
Mean (SD)	0.450	0.575 (0.5328)		
Median (Min, Max)	0.450 (0.45, 0.45)	0.575 (0.20, 0.95)		

N: number; SD: standard deviation; C_{max}: peak concentration; T_{max}: time to peak concentration; AUC_{0-t}: area under the plasma concentration-time curve from time 0 to last draw time; $t_{1/2}$: elimination half-life; λ_z: elimination rate constant.

Table 4. Summary of the number of lymph nodes detected

Detected lymph nodes	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=3)
In total				
Mean	48	46.7	60.3	49
Staining rate, (%)	21.0%	24.7%	32.5%	44.5%
Station 1				
Mean	42.3	44.3	57.3	44.3
Staining rate, (%)	23.2%	25.2%	34.1%	49.1%
Station 2				
Mean	5.7	2.3	3	4.7
Staining rate, (%)	0.0%	11.1%	0.0%	8.3%

Table 5. Summary of metastatic lymph nodes detected

Detected metastatic lymph nodes	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=3)
In total				
Mean	8.3	3	9.3	8.3
Staining rate, (%)	20.6%	36.1%	42.4%	21.0%
Station 1				
Mean	8	2.7	9	8
Staining rate, (%)	21.6%	25.0%	42.7%	31.5%
Station 2				
Mean	0.7	0.3	0.3	0.3
Staining rate, (%)	0.0%	100.0%	0.0%	0.0%

sis set. Two serious adverse events occurred in two subjects. No adverse reactions related to the study drug were recorded. No DLT was observed up to 14 ± 2 days post-drug, i.e., no toxic reactions of grade 3 or higher in blood or skin (**Tables 6** and **7**).

Discussion

Gastric cancer is prone to lymph node metastasis, especially in the locally progressive stage [19]. However, it is difficult to remove the lymph nodes completely because of their hidden location and difficulty in revealing [20]. The current tracers do not fully meet clinical needs. Mitoxantrone hydrochloride is an antineoplastic drug, mainly used in the treatment of malignant lymphoma [21, 22], breast cancer [23] and acute leukemia [24]. After modification,

Table 6. Adverse events in safety set

	1.0 ml group (N=3)		1.5 ml group (N=3)		2.0 ml group (N=3)		3.0 ml group (N=3)	
	No. of cases (%)	N	No. of cases (%)	N	No. of cases (%)	N	No. of cases (%)	N
Adverse Events	1 (33.3)	3	1 (33.3)	1	2 (66.7)	9	0 (0.0)	0
Adverse Reactions	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Serious Adverse Events	1 (33.3)	1	1 (33.3)	1	0 (0.0)	0	0 (0.0)	0
Serious Adverse Reactions	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Adverse events leading to dislodgment	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Severity (adverse events)	1 (33.3)	3	1 (33.3)	1	2 (66.7)	9	0 (0.0)	0
Grade 1	1 (33.3)	2	0 (0.0)	0	2 (66.7)	8	0 (0.0)	0
Grade 2	0 (0.0)	0	0 (0.0)	0	1 (33.3)	1	0 (0.0)	0
Grade 3	1 (33.3)	1	1 (33.3)	1	0 (0.0)	0	0 (0.0)	0
Grade 4	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Grade 5	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Severity (adverse reactions)	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Grade 1	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Grade 2	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Grade 3	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Grade 4	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Grade 5	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0

Number of cases: the number of subjects who had an adverse event; Number: the number of adverse events that occurred. If a subject has multiple adverse events during drug administration, the number of adverse events is counted only once for the agent used, but the number of cases for the agent used is counted separately.

Table 7. DLT assessment of patients in safety set [n (%)]

DLT Type	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=3)
Toxic reactions of grade 3 or higher in blood	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Toxic reactions of grade 3 or higher in the skin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

DLT: dose limiting toxicity.

MHI possess a strong lymphatic targeting ability, and notably, it does not enter the blood circulation, with low systemic effects and high safety. Pharmacokinetic data showed that the absorption of MHI was very low and that each subject had up to 4 concentration data exceed BQL. The maximum C_{max} detected was 37.4 ng/mL, which was only 7% of the C_{max} (510 ± 206 ng/mL) reported [25] when mitoxantrone was administered for chemotherapy (10-12 mg/m^2/d). The AUC reported [26] was 33600-102000 ng*min/mL when mitoxantrone (20-25 mg/m^2) was applied as chemotherapy drug, which is 16-49 times higher than the data in this study (The maximum AUC in this trial was 2081 ng*min/mL). The reason for the difference is that conventional mitoxantrone is administered periodically and intravenously during chemotherapy. While MHI is a modified agent with local slow bolus injection during surgery and resection after surgery.

In this study, gastric wall and perigastric lymph nodes were stained blue with MHI. The lymph node staining rates of the four groups were 21.0%, 24.7%, 32.5%, and 44.5%, respectively. Staining rate increased gradually with higher doses. Giving 3.0 mL of MHI realized the highest tracing effect, facilitating intraoperative exploration of more lymph nodes. It's also found that the staining rates of metastatic lymph nodes were above 20% in all four groups, suggesting that MHI also has a good lymph node staining effect for metastatic lymph nodes. All the stained lymph nodes did not fade

by the end of the operation. In the safety evaluation, no DLT and significant drug-related adverse reactions were observed with MHI, suggesting that 1.0-3.0 mL of this tracer is safe and generally does not cause systemic toxicities.

This trial has some limitations, including: (1) the sample size of this trail was small, and the exact efficacy needs to be further validated in large-sample phase II and III trails; (2) long-term adverse effects were not observed; (3) the duration of lymph node dissection in this study ranged from 30 to 45 min, and all the stained lymph nodes did not fade by the end of the operation. Whether this tracer can meet the application for longer surgeries needs to be further verified in later studies.

In conclusion, this study has highlighted the potential of Mitoxantrone Hydrochloride Injection (MHI) as a novel lymphatic tracer for patients undergoing surgery for gastric cancer. MHI exhibits strong lymphatic targeting capabilities, efficiently staining lymph nodes blue while minimally entering the bloodstream, thus demonstrating both effective lymphatic delineation and a favorable safety profile.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Summary of patients enrolled in full analysis set [n (%)*]

Analysis set	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=3)	All (N=12)
FAS	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	12 (100.0)
Safety set	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	12 (100.0)
PKCS	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	12 (100.0)
PKPS	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	12 (100.0)

Note: *: Percentages are calculated based on the number of subjects in the full analysis set as the denominator. FAS: full analysis set; PKCS: pharmacokinetics analysis concentration set; PKPS: pharmacokinetics analysis parameter set.

Supplementary Table 2. Summary of Protocol Deviations in full analysis set

Subject screening number	Type	Date of occurrence	Form	Violation/deviation from the program Event description
01001	Secondary	2020-09-04	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
01002	Secondary	2020-09-07	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
01003	Secondary	2020-09-21	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
		2020-10-08	Convenient routine	The date of routine stool examination is not within the (28 ± 3) d time window, exceeding the window by 1 day
01005	Secondary	2020-10-21	12-lead Electrocardiogram	Screening date is not within the time window and is the same day as the surgery day (protocol specifies screening period D-28D-1 and surgery day D1)
			Coagulation tests	Screening date is not within the time window and is the same day as the surgery day (protocol specifies screening period D-28D-1 and surgery day D1)
			Blood Biochemistry	Screening date is not within the time window and is the same day as the surgery day (protocol specifies screening period D-28D-1 and surgery day D1)
		2020-11-03	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
01006	Secondary	2020-10-26	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
		2020-11-21	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
01007	Secondary	2020-11-21	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
01008	Secondary	2020-11-20	Intraoperative cytology	Intraoperative cytology was not performed, and the investigator determined that it was not necessary
		2020-11-23	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
		2020-12-02	Convenient routine	Subjects did not defecate and did not undergo routine stool examination
01010	Secondary	2021-04-28	Intraoperative cytology	Intraoperative cytology was not performed, and the investigator determined that it was not necessary
01011	Secondary	2021-04-29	Intraoperative cytology	Intraoperative cytology was not performed, and the investigator determined that it was not necessary
		2021-05-06	Blood Biochemistry	Total cholesterol, triglycerides and alkaline phosphatase in the blood biochemical examination exceeded the window, should be May 1, 2021, the actual examination date is May 6, 2021
01014	Secondary	2021-05-24	Intraoperative cytology	Intraoperative cytology was not performed, and the investigator determined that it was not necessary

Supplementary Table 3. Subject distribution in screening set [n (%)]*

Test completion and reasons	1.0 ml group (N=3)	1.5 ml group (N=3)	2.0 ml group (N=3)	3.0 ml group (N=4)	All (N=13)
Selection					14
Not included					1
Joined	3 (100.0)	3 (100.0)	3 (100.0)	4 (100.0)	13 (100.0)
Complete	3 (100.0)	3 (100.0)	3 (100.0)	3 (75.0)	12 (92.3)
Withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (7.7)
Reasons for withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (7.7)
Subject withdrew voluntarily	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Poor compliance	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Intraoperative discrepancy with preoperative judgment	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
The occurrence of drug allergies and adverse reactions	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subject needs to withdraw from the trial	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (7.7)
Wrong dose or method of administration	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other serious protocol violations	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects missing interviews	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: *: Percentages are calculated based on the number of enrolled subjects as the denominator. N = number of subjects in each group who received a test number. Screening set: all subjects who signed the informed consent form.