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ORIGINAL ARTICLE

Amrubicin for the treatment of neuroendocrine carcinoma of the gastrointestinal tract: a retrospective analysis of five cases

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Abstract
Purpose A standard chemotherapy regimen for neuroendocrine carcinoma of the gastrointestinal tract (GI-NEC) has not been established. Treatment usually consists of platinum doublets, consistent with the standard treatment for small-cell lung cancer (SCLC), with which it shares clinicopathological similarities. Here, we retrospectively examined responses of five GI-NEC patients treated with amrubicin chloride (AMR) which has shown activity against SCLC as salvage therapy.
Methods Five patients with histologically proven unresectable GI-NEC in whom previous chemotherapy regimens had failed were treated with AMR, a synthetic anthracycline with potent topoisomerase II inhibition.
Results Primary tumors were located in the esophagus in three patients, anus in one, and colon in one. AMR was administered intravenously at 35–40 mg/m^2 on days 1–3 every 3 weeks for a median of six treatment cycles (range, 2–8). Although all patients had received one to four previous chemotherapy regimens, including cisplatin doublets, three of five achieved objective responses to AMR. All three had esophageal NEC in relapse following combination treatment with irinotecan plus cisplatin. The most common adverse events of $\geq$ grade 3 were neutropenia (75%), anemia (60%), thrombocytopenia (20%), and febrile neutropenia (20%).
Conclusions Single-agent AMR achieved objective responses in three of five patients with GI-NEC. This compound may be a candidate for prospective evaluation in a larger series.

Keywords Gastrointestinal tract · Small-cell carcinoma · Neuroendocrine carcinoma · Chemotherapy · Amrubicin

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Introduction

Neuroendocrine carcinoma (NEC) of the gastrointestinal (GI) tract (GI-NEC) is an uncommon disease, which accounts for 0.1–1.0% of all GI malignancies. This sparsity, together with the lack of any universally accepted nomenclature, has hampered understanding of this disease and the development of standard treatments. Previous papers have variously referred to anaplastic [1], poorly differentiated [2–4], and high grade [5–7] neuroendocrine carcinoma or small-cell carcinoma (SCC) [8–11], in an almost synonymous manner. However, these classifications were made on the basis of different diagnostic criteria, and it remains unclear which is most appropriate for the diagnosis of neuroendocrine carcinoma, particularly for tumors with atypical features of SCC. The most recent WHO classification, in 2010, attempts to overcome this difficulty by proposing the use of the widely accepted term NEC, which it defined as a poorly differentiated, high-grade malignant neoplasm with diffuse expression of neuroendocrine markers, no matter whether of the small- or large-cell type [12]. We have used this definition of GI-NEC in the present paper.

GI-NEC has an aggressive natural history that is characterized by early, widespread metastasis, and at least half of all patients have overt distant metastases at diagnosis [13]. Due to the very low incidence of the disease,

however, no standard chemotherapy regimen has yet been established. The National Comprehensive Cancer Network (NCCN) guideline recommends adopting chemotherapeutic regimens for small-cell lung cancer (SCLC) for the treatment of metastatic GI-NEC.

Amrubicin chloride (AMR) is a synthetic anthracycline, which shows potent topoisomerase II inhibition. This agent has shown activity against SCLC and was approved for use in Japan in 2002. Given that the NCCN guideline recommends the use of SCLC therapeutic regimens for GI-NEC, we retrospectively evaluated the responses of five GI-NEC patients treated with AMR and reviewed the literature. The present study was performed under an institutional review board (IRB) waiver from the IRB chairperson and the written authorization of the chief director of the hospital, in accordance with the Japanese ethical guidelines for epidemiological research [14].

Materials and methods

Of 21 patients diagnosed with unresectable GI-NEC at our institution between January 2002 and December 2008, 5 received an intravenous AMR regimen after the failure of previous chemotherapy regimens. For each patient, administration of AMR was agreed to by the attending oncologist and the patient himself or herself. Here, we retrospectively reviewed the medical records of these five subjects.

Results

Patient characteristics

Patient characteristics at the start of AMR therapy are shown in Table 1. The histological diagnosis of NEC was made using surgical specimens for Patients 2, 3, and 5 and biopsy specimens for Patients 1 and 4, in accordance with the WHO classification (2010) [12]. The diagnosis of NEC was confirmed by diffusely positive immunohistochemical reactions to at least one of chromogranin A, CD56, or synaptophysin. Tumors were strongly positive to Ki67 in all patients, except those of Patient 4. Histologically, Patient 3 had anal cancer composed of 90% NEC and 10% adenocarcinoma, while the others showed NEC only. All patients had received one to four chemotherapy regimens prior to AMR, including platinum doublets, and had radiographically documented progression or relapse of disease.

Treatment results

AMR was administered as a 5-min injection at a dose of 40 mg/m^2 on days 1–3 every 3 weeks, which is a recommended salvage therapy dosage for SCLC. Dosage was reduced to 35 mg/m^2 in Patients 4 and 5 because of poor performance status (Table 2). Premedication with 1 mg of granisetron and 8 mg of dexamethasone sodium phosphate was administered to all patients. The median number of

Table 1 Patient characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	60	60	70	60	61
Sex	M	M	F	M	M
PS (ECOG)	0	1	0	2	2
Primary site	Esophagus	Esophagus	Anus	Colon	Esophagus
Histological diagnosis	NEC	NEC	NEC + adenocarcinoma	NEC	NEC
IHC					
CD56 (NCAM)	3+	3+	3+	+	–
CGA	–	–	3+	+	–
Synaptophysin	1+	3+	3+	+	3+
Ki67 index (%)	80	60	60	NE	60
Metastatic/recurrent	Metastatic	Recurrent	Recurrent	Metastatic	Recurrent
Metastatic sites	LN, Lung Liver, Bone	LN	Lung	LN, Bone	LN, Brain, Peritoneum
Prior chemotherapy	IP	IP	1. IP	EP	1. IP
			2. UFT + LV		2. WBRT
			3. FOLFOX		3. DTX
			4. S-1 + aflibercept		

PS performance status; *ECOG* Eastern Cooperative Oncology Group; *NEC* Neuroendocrine carcinoma; *IHC* immunohistochemistry; *NCAM* neural cell adhesion molecule; *CGA* chromogranin A; *NE* not examined; *LN* lymph node; *IP* irinotecan + cisplatin; *UFT* tegafur uracil; *LV* leucovorin; *EP* etoposide + cisplatin; *WBRT* whole brain radiation therapy; *DTX* docetaxel

Table 2 Treatment intensity

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
AMR dose (mg/m^2)	40	40	40	35	35
No. of cycles	5	8	6	2	6
Dose intensity (mg/m^2/week)	30.7	32	26.8	31	24.2
Relative dose intensity (%)	75.4	80	67	77.5	60.5

Relative dose intensity was calculated as the ratio of actual dose intensity to planned dose intensity (40 mg/m^2/week)

AMR amrubicin chloride

treatment cycles was six (range, 2–8). Treatment delays occurred in all patients, and Patients 1, 3, and 5 required dose reductions because of severe hematological toxicity. The median relative dose intensity (RDI), namely the ratio of actual to planned dose intensity (40 mg/m^2/week) was 75.4% (range, 60.5–80%). RDIs were low in Patients 3 and 5, with histories of four and two previous therapeutic regimens, respectively (<70%, Table 2).

Adverse events during AMR treatment are shown according to Common Terminology Criteria for Adverse Events ver. 3.0 in Table 3. Excluding Patient 2, all patients experienced grade 3 or worse neutropenia after the first or second administration. Three patients experienced grade 3 or 4 anemia, and Patient 1 experienced grade 3 thrombocytopenia. Nonhematologic toxicities, mainly anorexia, nausea, and diarrhea, were all grade 1 or 2 except one case of febrile neutropenia. Tumor response was measured based on Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.0 by computed tomography (CT) scanning without interval confirmation (Table 4). Three patients achieved objective responses, with durations ranging from 75 to 122 days (Patients 1, 2, and 5). All three had esophageal NEC with evidence of refractoriness to the combination of irinotecan and cisplatin. Patient 5 achieved not only tumor regression but also symptom relief. At the initiation of AMR therapy, this patient had massive lymph node swelling in the abdominopelvic cavity and suffered from severe edema of the lower extremities due to venous stasis and iliofemoral thrombosis. Edema disappeared after two cycles of AMR, and CT scanning showed shrinkage of the lymph node metastases and the regression of thrombosis (Fig. 1).

All patients died of disease progression. Median survival after the initiation of AMR therapy was 217 days (range, 107–594 days). Disease progression following AMR therapy was treated with etoposide plus cisplatin, oral etoposide, or irinotecan plus cetuximab, none of which produced an objective response.

Table 3 Adverse events

Adverse event	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Hematologic					
Leukopenia	3	1	3	3	4
Neutropenia	3	1	4	3	4
Anemia	3	1	2	4	3
Thrombocytopenia	3	1	1	1	2
Nonhematologic					
Anorexia	2	2	1	0	0
Nausea	1	0	1	0	0
Diarrhea	0	1	1	0	1
Constipation	0	0	1	0	0
Fever	0	1	0	0	0
Febrile neutropenia	3	0	0	0	0

All adverse events were graded as the worst observed grade, according to the Common Terminology Criteria for Adverse Events ver. 3.0

Discussion

Here, we report our experience with AMR treatment in five GI-NEC patients as salvage therapy. Although all patients had been intensively treated with previous chemotherapy regimens, three with esophageal NEC achieved an objective response. Although patient number was low, these findings indicate the potential usefulness of AMR in the treatment of this uncommon tumor.

Many investigators have recommended that GI-NEC be managed in a similar manner to SCLC, based on the clinicopathological similarities between them [13]. Under light microscopy, the small-cell type of GI-NEC shows the appearance of SCLC, although the large-cell type has more abundant cytoplasm, visible nucleoli, and vesicular chromatin [7]. NEC may contain non-NEC components in part, such as adenocarcinoma and squamous cell carcinoma [6, 10–13]. Its neuroendocrine features are indicated by immunohistochemical staining for neuroendocrine markers, such as chromogranin A, synaptophysin, and CD56 (NCAM) [7]. GI-NEC also has clinical similarities to SCLC, such as high metastatic potential, high chemosensitivity, albeit of short duration, and overall poor prognosis [13]. The median survival of patients with unresectable GI-NEC treated with the combinations of etoposide/cisplatin (EP), irinotecan/cisplatin (IP), and paclitaxel/carboplatin/etoposide has been reported to range from 7 to 19 months [1–5, 8, 9].

Standard first-line regimens for metastatic SCLC include platinum-based chemotherapies, such as EP [15]

Table 4 Efficacy of AMR treatment for GI-ECC

	Case 1	Case 2	Case 3	Case 4	Case 5
Best response	PR	CR	SD	PD	PR
Treatment effect duration (day)	75	122	87	–	114
PFS (day)	138	299	162	41	174
OS (day)	217	594	454	107	210
Treatment after progression	1. EP	1. EP	1. Oral ETP	–	–
	2. WBRT	2. WBRT	2. Palliative RT		
			3. Irinotecan + Cetuximab		

PR partial response; *CR* complete response; *SD* stable disease; *PD* progressive disease; *EP* etoposide plus cisplatin; *WBRT* whole brain radiation therapy; *ETP* etoposide; *RT* radiation therapy

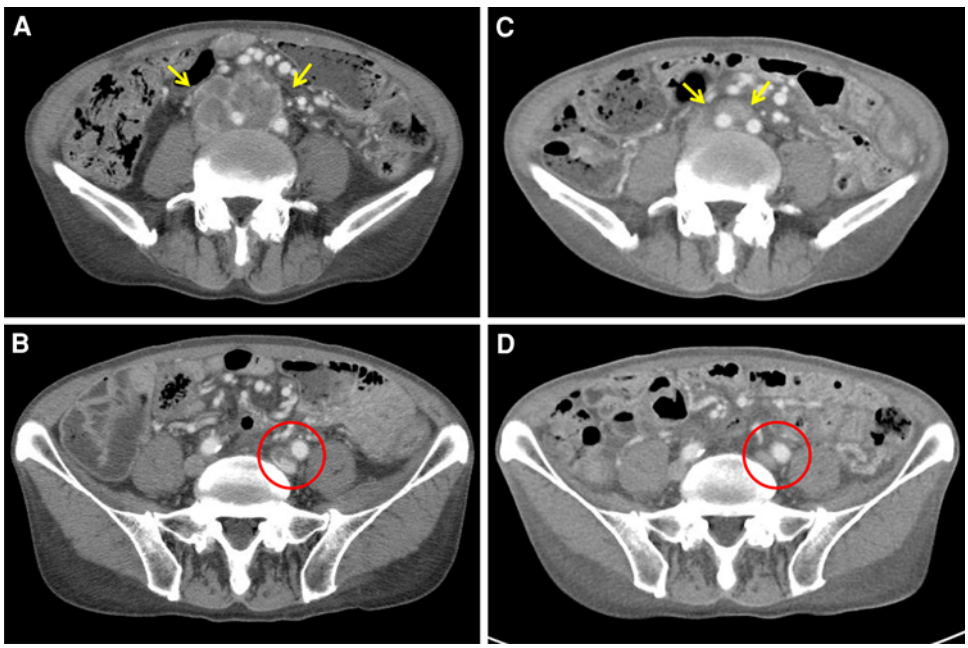

Fig. 1 CT images of lymph node metastases in Patient 5. Before AMR therapy, massive enlargement of abdominopelvic lymph nodes (**a**) resulted in iliofemoral thrombosis. **b** After three cycles of AMR, lymph node metastases showed significant shrinkage (**c**), and the thrombosis disappeared (**d**)

and IP [16]. The strongest predictor of outcome for patients with relapsed disease is considered to be the duration of remission with the initial therapy. These patients are distinguished as having "sensitive-relapse" (e.g., remission extends beyond 3 months) or "refractory-relapse" (disease progresses within 3 months) SCLC. Topotecan is approved as salvage therapy for sensitive-relapse SCLC by the US Food and Drug Administration based on a response rate (RR) of 24% and a median overall survival (MST) of 6 months in this population [17]. However, results in refractory-relapse SCLC have been unsatisfactory, with RRs of only 0 and 12% [17, 18]. Recent data from phase II trials suggest that AMR has promising activity in refractory SCLC, with RRs of 17–52.9% [19–21] and an MST of 6 months [21]. These findings provide the rationale for our selection of AMR as salvage therapy for NEC.

In our study, the outcome of AMR treatment did not reflect the response to initial chemotherapy when the initial treatment was IP. Patients 1 and 2, who relapsed during adjuvant IP, a situation we term refractory relapse, showed good tumor regression after AMR administration. On the other hand, Patient 4 was unresponsive to both EP and subsequent AMR treatment. Etoposide works as a topoisomerase-2 inhibitor and is a substrate of P-glycoprotein (P-gp), a transmembrane ATP-dependent efflux pump of various xenobiotic compounds. P-gp mediates the cellular mechanism of multidrug resistance by decreasing intracellular drug concentration [22]. Additionally, alterations in topoisomerase-2 have been implicated in resistance to etoposide. The target of AMR is also topoisomerase-2, albeit that no data are available on its possible induction of drug resistance. Recent in vitro studies have implied that AMR is also a substrate for P-gp [23, 24]. We speculate that P-gp might have contributed to resistance to both regimens in Patient 4.

To our knowledge, three case reports have described extra-pulmonary SCC responsive to AMR to date, involving the prostate [25], liver [26], and thymus [27]. The

former two patients received etoposide-containing regimens as well. The patient with liver SCC demonstrated a good response to EP [26], while bone metastases of prostate SCC were unresponsive to etoposide/carboplatin [27]. Although the information available is thus markedly limited, we believe that AMR might be a candidate for a clinical trial in GI-NEC.

Several limitations of the study warrant mention. First, the study was conducted under a retrospective design in only a small number of patients at a single institution. The demonstrated efficacy of AMR should therefore be considered with caution and will require confirmation in a larger prospective series. Second, the patients were quite heterogeneous, with different tumor origins and different treatment histories. Nevertheless, they represent an important addition to the marked paucity of information on salvage therapy for GI-NEC to date. We speculate that esophageal NEC refractory to IP might be a candidate for further evaluation, and given the new diagnostic criteria and unified nomenclature proposed by WHO, the accumulation of small experiences such as the present series should lead to the establishment of standard treatment for this uncommon disease.

As has also been reported with SCLC, the most frequent and severe adverse event was bone marrow suppression, which necessitated a treatment delay or dose reduction. Most events occurred during the first or second course. Four phase II trials which included previously treated SCLC patients reported that 40 mg/m^2 AMR on days 1–3 caused grade 3 or 4 neutropenia, thrombocytopenia, and anemia in 42–93%, 18–40.6%, and 20–33% of patients, respectively [18, 20, 21, 28]. With regard to nonhematological toxicities, while we observed digestive symptoms such as anorexia and diarrhea, all cases were moderate.

In conclusion, AMR achieved an objective response in three of five GI-NEC patients evaluated in this study, with manageable toxicities. These results warrant the further prospective investigation of this agent for GI-ECC.

Conflict of interest None.

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