TABLE 4. Overall survival and local control rates by local extension site.

	Invasion	No. of patients	5-y OS, %	*p* value	5-y LC, %	*p* value
Superomedial						
Middle nasal meatus	+	93	43.4	.066	42.8	**.033**
	−	25	75.4		71.2	
Ethmoid sinuses	+	86	40.0	**.014**	43.0	.078
	−	32	75.9		64.4	
Cribriform plate	+	26	19.1	**.033**	39.6	.240
	−	92	56.8		51.6	
Frontal sinus	+	13	58.3	.690	69.2	.211
	−	105	48.7		46.7	
Dura	+	16	16.9	**.001**	8.2	**< .001**
	−	102	54.7		54.7	
Superior						
Anterior orbital contents	+	87	47.8	.836	49.1	.898
	−	31	57.9		48.7	
Orbital apex	+	28	30.4	.087	35.7	.124
	−	90	56.8		52.9	
Posterior						
Posterior wall	+	95	49.8	.432	50.9	.329
	−	23	49.4		41.0	
Pterygoid fossa	+	69	44.7	.636	46.1	.687
	−	49	56.9		53.2	
Pterygoid plates	+	71	42.0	.102	42.2	.128
	−	47	62.4		58.7	
Sphenoid sinus	+	30	34.4	**.021**	36.4	.134
	−	88	55.9		53.3	
Nasopharynx	+	13	12.5	**.002**	21.0	.090
	−	105	54.6		52.1	
Middle cranial fossa	+	20	23.2	**.013**	24.9	.179
	−	98	55.9		53.3	
Lateral						
Infratemporal fossa	+	70	49.4	.840	46.5	.536
	−	48	50.0		52.0	
Inferior						
Hard palate	+	48	30.1	.057	36.8	**.027**
	−	70	59.3		56.9	
Anterior						
Subcutaneous tissue	+	78	47.5	.771	49.5	.936
	−	40	56.0		47.8	
Skin of cheek	+	19	39.3	.882	59.1	.370
	−	99	51.5		47.1	
Cranial nerve						
Other than V2	+	10	11.7	**.010**	11.7	.065
	−	108	53.6		52.4	

Abbreviations: OS, overall survival; LC, local control.
The *p* values in boldface represent < .05.

pterygomaxillary fossa or frontal and sphenoid sinuses, erosion of the cribriform plate, and invasion of the dura had a significantly worse prognosis. Although the target disease and anatomic sites analyzed did not necessarily accord with those in our study, their results were similar to our results. The reason for this is thought to be that the superomedial and posterior sites are more difficult to access for tumor resection while ensuring adequate surgical margins. These results suggested that further additional treatment is required for tumors that progress deeply in the superomedial and posterior directions.

Interestingly, there were statistically significant differences in the overall survival and local control rates among patients with invasion into the middle nasal meatus and ethmoid sinuses. Patients with tumors not invading the middle nasal meatus or ethmoid sinuses had better 5-year overall survival rates of 75.4% and 75.9%, respectively. The reason for this was considered to be that tumors not progressing into these sites did not invade more deeply into areas such as the cribriform plate or dura and, therefore, complete resection of such tumors was relatively easy. However, there was no significant correlation between these sites.

Additionally, patients with tumor extension into the hard palate, which is a factor defined as T2 disease, had an unexpectedly poor prognosis. This site is not so difficult to resect completely; however, our results showed that invasion into this site was related to invasion toward posterior sites, such as the middle cranial fossa. Furthermore, the hard palate was the only site among those

TABLE 5. Correlations among local extension and poor prognostic sites or neck lymph node metastasis.

	Cribriform plate	Dura	Nasopharynx	Middle cranial fossa	Other than V2	Lymph node metastasis
Middle nasal meatus	0.996	0.558	0.366	0.296	0.616	0.925
Ethmoid sinuses	0.076	0.266	0.986	0.966	0.557	0.776
Cribriform plate	N/A	**0.009** (r = 0.267)	**0.009**(r = 0.270)	0.955	0.813	0.709
Frontal sinus	**<0.001** (r = 0.401)	0.527	0.316	0.581	0.525	0.486
Dura	**0.009** (r = 0.267)	N/A	**0.018** (r = 0.256)	**<0.001** (r = 0.481)	0.269	0.738
Anterior orbital contents	0.868	0.099	0.955	0.891	0.924	0.089
Orbital apex	**<0.001** (r = 0.328)	**0.003** (r = 0.303)	0.328	0.312	**0.015** (r = 0.259)	0.339
Posterior wall	0.809	0.796	0.443	0.805	0.708	0.899
Pterygoid fossa	0.588	0.532	**0.003** (r = 0.297)	0.058	**0.014** (r = 0.256)	0.760
Pterygoid plates	0.872	**0.033** (r = 0.221)	**0.027** (r = 0.231)	**0.006** (r = 0.275)	**0.019** (r = 0.248)	0.542
Sphenoid sinus	**<0.001** (r = 0.441)	**0.001** (r = 0.337)	**<0.001** (r = 0.354)	**0.013** (r = 0.255)	0.974	0.553
Nasopharynx	**0.009** (r = 0.270)	**0.018** (r = 0.256)	N/A	**<0.001** (r = 0.418)	0.674	0.117
Middle cranial fossa	0.955	**<0.001** (r = 0.481)	**<0.001** (r = 0.418)	N/A	0.111	0.264
Infratemporal fossa	0.794	0.581	0.899	0.188	0.291	0.088
Hard palate	0.848	0.996	0.054	**0.029** (r = 0.224)	0.335	**0.007** (r = 0.268)
Subcutaneous tissue	**<0.001** (r = −0.354)	0.541	0.954	0.885	0.141	0.983
Skin of cheek	0.308	0.956	0.745	0.631	0.318	0.208
Other than V2	0.813	0.269	0.674	0.111	N/A	0.757

Abbreviations: r, φ correlation coefficient; N/A, not available.
The figures in boldface represent p value $< .05$.

TABLE 6. Initial treatment for patients with tumor extension into poor prognostic sites.

		No. of patients (%)				
		Total maxillectomy	Partial maxillectomy	RADPLAT	IV-CRT	Others
Cribriform plate	26	6 (23)	4 (15)	3 (12)	7 (27)	6 (23)
Dura	16	2 (13)	1 (6)	5 (31)	6 (38)	2 (13)
Nasopharynx	13	1 (8)	3 (23)	3 (23)	3 (23)	3 (23)
Middle cranial fossa	20	3 (15)	4 (20)	7 (35)	4 (20)	2 (10)
Other than V2	10	2 (20)	3 (30)	3 (30)	2 (20)	0
Any of the 5 poor prognostic sites	48	9 (19)	11 (23)	10 (21)	11 (23)	7 (15)

Abbreviations: RADPLAT, radiation and intra-arterial cisplatin; IV-CRT, intravenous chemotherapy with concomitant radiotherapy.

examined in this study that was correlated with neck lymph node metastasis. Although the risk of lymph node metastasis is relatively low in maxillary sinus cancer, patients with nodal involvement demonstrated a worse prognosis.[10,13] Several reports have shown that patients with T2 primary tumors of the maxillary sinus exhibit high rates of regional failure.[14,15] The reason for this was speculated to be that the mucosa of the hard palate have a more extended lymphatic network than the mucosa of the maxillary sinus. Therefore, with regard to nodal involvement, tumors extending into the hard palate behave more like oral cancers than other maxillary sinus cancers. The poor prognosis of patients with tumors extending into the hard palate might be related to the high risk of lymph node metastasis.

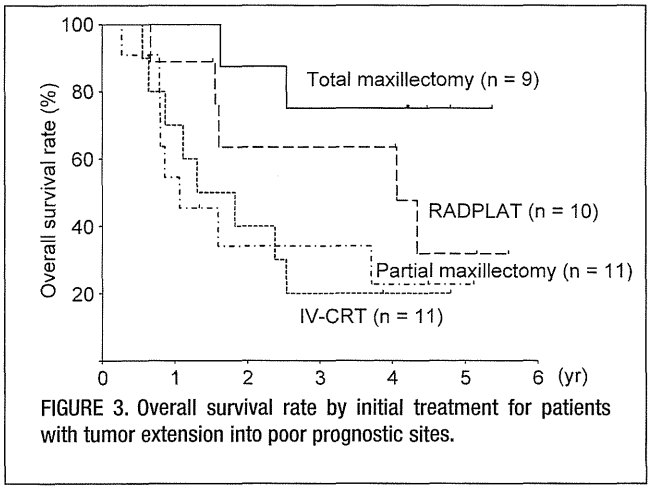

FIGURE 3. Overall survival rate by initial treatment for patients with tumor extension into poor prognostic sites.

The cranial nerve other than V2 was the only independent factor among the poor prognostic sites. However, it showed significant correlations with the orbital apex, pterygoid fossa, and pterygoid plates. The II, III, IV, V1, and V3 cranial nerves pass through the superior orbital fissure or pterygopalatine fossa.[16] Therefore, tumors invading the orbital apex, pterygoid fossa, or pterygoid plates would induce the involvement of those cranial nerves.

Regarding the treatment for tumors extending into such poor prognostic sites, our results suggested that if complete resection of tumor was possible, total maxillectomy

would be desirable. Of 9 patients treated with total maxillectomy, 5 patients simultaneously underwent anterior and middle skull base resection and 1 patient underwent middle skull base resection. Three of the patients had no recurrence of primary tumor. RADPLAT would be more suitable than other treatments for patients in whom radical surgery, including complete resection of the contents of the orbit or skull base surgery was not possible. We have reported that the 5-year local progression-free survival and overall survival rates were 69.0% and 61.1%, respectively, for patients with T4b disease treated with RADPLAT.[9]

In conclusion, poor survival rates and local failure were closely related to anatomic tumor extension site, and the cribriform plate, dura, nasopharynx, middle cranial fossa, and cranial nerves other than V2, in particular, were identified as poor prognostic sites. The hard palate was the only site among those examined in this study found to be correlated to nodal involvement and showed a poor treatment outcome. Even in cases with similar T4 SCCs in the maxillary sinus, treatment should be performed in consideration of the local extension sites.

Acknowledgments

This study was supported in part by a Health and Labour Sciences Research Grant for Clinical Cancer Research (H22-Gannrinshou-Ippan-017) from the Ministry of Health, Labour and Welfare of Japan, the National Cancer Center Research and Development Fund (23-A-21) of Japan, and a grant-in-aid for Scientific Research (C) (KAKENHI 24592587) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

APPENDIX

In addition to the authors, the following investigators participated in this study: *Aichi Medical University, Nagakute*—A. Ikeda; *Iwate Prefectural Central Hospital, Morioka*—S. Kato; *Kanagawa Cancer Center, Yokohama*—A. Kubota; *Kyoto Prefectural University of Medicine, Kyoto*—K. Ikebuchi; *Kochi Health Sciences Center, Kochi*—K. Kozakura; *Kobe University Graduate School of Medicine, Kobe*—K. Nibu; *Jichi Medical University, Shimotsuke*—H. Nishino; *Jikei University School of Medicine, Tokyo*—T. Kato; *Tokyo University Graduate School of Medicine, Tokyo*—T. Asakage; *Japanese Red Cross Nagoya Daiichi Hospital, Nagoya*—K. Kawata; *Nara Medical University, Kashihara*—I. Ota; *Hiroshima University Hospital, Hiroshima*—T. Ueda; *Keiyukai Sapporo Hospital, Sapporo*—A. Watanabe; *Kyoto University Graduate School of Medicine, Kyoto*—M. Kitamura.

REFERENCES

1. Japan Society for Head and Neck Cancer. Report of Head and Neck Cancer of Japan Clinical Statistics of Registered Patients, 2003. *Jpn J Head Neck Cancer* 2007;33(Suppl):90–96.
2. Sato Y, Morita M, Takahashi HO, Watanabe N, Kirikae I. Combined surgery, radiotherapy, and regional chemotherapy in carcinoma of the paranasal sinuses. *Cancer* 1970;25:571–579.
3. Nishino H, Ichimura K, Tanaka H, et al. Results of orbital preservation for advanced malignant maxillary sinus tumors. *Laryngoscope* 2003;113:1064–1069.
4. Al-Sarraf M, Pajak TF, Marcial VA, et al. Concurrent radiotherapy and chemotherapy with cisplatin in inoperable squamous cell carcinoma of the head and neck. An RTOG Study. *Cancer* 1987;59:259–265.
5. Merlano M, Benasso M, Corvò R, et al. Five-year update of a randomized trial of alternating radiotherapy and chemotherapy compared with radiotherapy alone in treatment of unresectable squamous cell carcinoma of the head and neck. *J Natl Cancer Inst* 1996;88:583–589.
6. Paccagnella A, Orlando A, Marchiori C, et al. Phase III trial of initial chemotherapy in stage III or IV head and neck cancers: a study by the Gruppo di Studio sui Tumori della Testa e del Collo. *J Natl Cancer Inst* 1994;86:265–272.
7. Kanoto M, Oda A, Hosoya T, et al. Impact of superselective transarterial infusion therapy of high-dose cisplatin on maxillary cancer with orbital invasion. *AJNR Am J Neuroradiol* 2010;31:1390–1394.
8. Robbins KT. The evolving role of combined modality therapy in head and neck cancer. *Arch Otolaryngol Head Neck Surg* 2000;126:265–269.
9. Homma A, Oridate N, Suzuki F, et al. Superselective high-dose cisplatin infusion with concomitant radiotherapy in patients with advanced cancer of the nasal cavity and paranasal sinuses: a single institution experience. *Cancer* 2009;115:4705–4714.
10. Bhattacharyya N. Factors affecting survival in maxillary sinus cancer. *J Oral Maxillofac Surg* 2003;61:1016–1021.
11. Airoldi M, Garzaro M, Valente G, et al. Clinical and biological prognostic factors in 179 cases with sinonasal carcinoma treated in the Italian Piedmont region. *Oncology* 2009;76:262–269.
12. Dulguerov P, Jacobsen MS, Allal AS, Lehmann W, Calcaterra T. Nasal and paranasal sinus carcinoma: are we making progress? A series of 220 patients and a systematic review. *Cancer* 2001;92:3012–3029.
13. Carrillo JF, Güemes A, Ramírez–Ortega MC, Oñate–Ocaña LF. Prognostic factors in maxillary sinus and nasal cavity carcinoma. *Eur J Surg Oncol* 2005;31:1206–1212.
14. Cantù G, Bimbi G, Miceli R, et al. Lymph node metastases in malignant tumors of the paranasal sinuses: prognostic value and treatment. *Arch Otolaryngol Head Neck Surg* 2008;134:170–177.
15. Morris LG, Patel SG, Shah JP, Ganly I. High rates of regional failure in squamous cell carcinoma of the hard palate and maxillary alveolus. *Head Neck* 2011;33:824–830.
16. McMahon JD, Wong LS, Crowther J, et al. Patterns of local recurrence after primary resection of cancers that arise in the sinonasal region and the maxillary alveolus. *Br J Oral Maxillofac Surg* 2013;51:389–393.

Eur Arch Otorhinolaryngol (2014) 271:2767–2770
DOI 10.1007/s00405-013-2806-4

HEAD AND NECK

The incidence of late neck recurrence in N0 maxillary sinus squamous cell carcinomas after superselective intra-arterial chemoradiotherapy without prophylactic neck irradiation

Tomohiro Sakashita · Akihiro Homma · Hiromitsu Hatakeyama · Satoshi Kano · Takatsugu Mizumachi · Jun Furusawa · Daisuke Yoshida · Noriyuki Fujima · Rikiya Onimaru · Kazuhiko Tsuchiya · Koichi Yasuda · Hiroki Shirato · Satoshi Fukuda

Received: 18 September 2013 / Accepted: 30 October 2013 / Published online: 9 November 2013

Abstract The efficacy of elective neck irradiation (ENI) for patients with N0 carcinoma of the maxillary sinus has been controversial. The purpose of our study was to investigate the incidence of late neck recurrence and the mortality rate from regional disease in patients with N0 maxillary sinus cancer after superselective cisplatin infusion and concomitant radiotherapy (RADPLAT) without ENI. We retrospectively analyzed 48 patients with N0 maxillary sinus cancer who underwent RADPLAT. Chemotherapy consisted of 100–120 mg/m^2 superselective intra-arterial cisplatin administered at a median rate of four times weekly. Concurrent radiation therapy was administered at a median dose of 65 Gy without ENI. Late neck recurrence was observed in 8.3 % (4/48). Three patients underwent salvage neck dissection and survived without any evidence of disease. The remaining patient did not undergo neck dissection due to coexistence with distant metastasis, and he died of regional disease. The mortality rate from regional disease was calculated to be 2 % (1/48). The incidence of late neck recurrence was not frequent, and the mortality rate from regional disease was low. Salvage neck dissection was considered to be feasible for patients with late neck recurrence. When definitive radiotherapy and concomitant chemotherapy are applied, it is considered that ENI is not required for cases of N0 maxillary sinus cancer.

T. Sakashita (✉) · A. Homma · H. Hatakeyama · S. Kano · T. Mizumachi · J. Furusawa · S. Fukuda
Department of Otolaryngology–Head and Neck Surgery, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan
e-mail: t-sakashita@med.hokudai.ac.jp

D. Yoshida · N. Fujima · R. Onimaru · K. Tsuchiya · K. Yasuda · H. Shirato
Department of Radiology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Keywords Maxillary sinus cancer · Elective neck irradiation · Intra-arterial chemotherapy · Radiotherapy · Regional recurrence

Introduction

Carcinomas of the maxillary sinus are uncommon, comprising just 0.2–0.5 % of all cancers, 3 % of all head and neck cancers, and 80 % of all paranasal sinus cancers [1]. Although nodal metastasis is uncommon in maxillary sinus cancer, many authors have advocated elective neck irradiation (ENI) based on the 10–29 % rate of neck recurrence and the poor survival of patients with recurrent disease in the neck [2, 3]. However, the role of ENI for patients with N0 maxillary sinus cancer remains controversial. In our institution, ENI has not been applied when patients with N0 maxillary sinus cancer undergo superselective cisplatin infusion and concomitant radiotherapy (RADPLAT). The purpose of our study was to investigate the incidence of late neck recurrence and the mortality rate from regional disease in patients with N0 maxillary sinus cancer after RADPLAT without ENI.

Method

Patients

We retrospectively analyzed 49 patients with N0 maxillary sinus squamous cell carcinomas who underwent RADPLAT in Hokkaido University Hospital, Japan between September 1999 and July 2012. One patient, who died of primary disease within 6 months after the end of treatment, was excluded. The remaining 48 patients were eligible for

Table 1 Patient demographics

Characteristic	No. of patients (%)
Total	48
Gender	
Male	38 (79 %)
Female	10 (21 %)
Age, years	
Median	60.5
Range	35–73 (ave. 58.7)
Follow-up period, months	
Median	69.5
Range	15.4–142.5 (ave. 62.1)
T classification	
T2	1 (2 %)
T3	14 (29 %)
T4a	23 (48 %)
T4b	10 (21 %)

our study. T and N stages were classified according to the American Joint Committee on Cancer (AJCC) staging system 2010. Table 1 presents patient demographics.

Approval for this study was obtained from the institutional review board at Hokkaido University. Completion of the survey was considered to represent implied consent for participation.

Radiotherapy

The irradiation plan during the period 2006–2012 was 70 Gy in 35 fractions of 2 Gy over 7 weeks for the primary site. Between 1999 and 2005, the primary site was irradiated with 65 Gy in 26 fractions of 2.5 Gy over 6.5 weeks. Elective irradiation of nodal area was not attempted.

Chemotherapy

Chemotherapy consisted of the administration of 100–120 mg/m^2 superselective intra-arterial cisplatin at a median rate of four times weekly (range 2–5 times, mean 3.7 times). At the same time, sodium thiosulfate was administered intravenously (24 g/body) to provide effective cisplatin neutralization.

Monitoring management

Patients were usually monitored monthly for recurrence in the first year, every couple of months in the second year, and every 6 or 12 months thereafter until death or data censoring. Ultrasonography and/or CT scan of the nodal area was routinely performed once in every 3 months in the first year, and every 6 or 12 months thereafter. If late neck recurrence was observed, salvage neck dissection was indicated.

Statistics

The Kaplan–Meier method was applied for overall survival. The time of interest was the duration from the start of the treatment to death or censoring. JMP Pro 10.0.2 statistical software (SAS Institute, Cary, NC, USA) was used for the analysis.

Results

Chemoradiotherapy

Intra-arterial chemotherapy was performed for a median of 4 times (range 1–5 times). The median irradiation dose was 70 Gy. The irradiation dose ranged from 48 to 70 Gy. Forty-five patients (93.7 %) underwent a full course of irradiation (>65 Gy).

Clinical outcomes of nodal status

The median follow-up period for living patients was 69.5 months (range 15.4–142.5 months). Late neck recurrence was observed in 8.3 % (4/48) at a median 11 months (range 1–32 months) after the completion of RADPLAT. Details of the four patients with late neck recurrence are given in Table 2. Neck dissection was performed in three of the four patients with late neck recurrence. These three patients survived without any evidence of disease. Neck dissection was not performed in the remaining patient due to coexistence with distant metastasis, and he died of regional disease 2 months after neck recurrence.

Five patients died of primary disease without any regional disease at a median 22 months (range 8–46 months) after the completion of RADPLAT. Two patients died of other disease without any regional disease 11–43 months after the completion of RADPLAT. Another patient died of distant disease without any regional disease 6 months after the completion of RADPLAT. The mortality rate from regional disease was calculated to be 2 % (1/48).

The 5-year overall survival rate of all 48 participants was calculated to be 78.2 % by the Kaplan–Meier method (Fig. 1).

Salvage neck dissection

Salvage neck dissection was performed in three patients. In one of these three patients, radiotherapy and chemotherapy were suspended at an irradiation dose of 50 Gy as an allergic reaction was observed after the second intra-

Table 2 Details of the four patients with late neck recurrence

Case	Age	Sex	T classification	Radiation dose	Chemotherapy (no. of times)	Extent of primary disease	Recurrent nodal area	Treatment for subsequent nodal metastasis	Outcome (observation period after recurrence)
1	45	F	4a	65	4	Infra-temporal fossa	IIa	Neck dissection	NED (9 years)
2	41	M	4b	65	4	Orbital apex	IIa	Palliative treatment	Dead of regional disease (2 months)
3	67	M	4a	50	2	Infra-temporal fossa and oral mucosa	Ib	Neck dissection associated with resection of the primary tumor	NED (5.7 years)
4	37	M	4a	70	4	Frontal wall and skin	Intra-parotid	Neck dissection	NED (5.5 years)

NED no evidence of disease

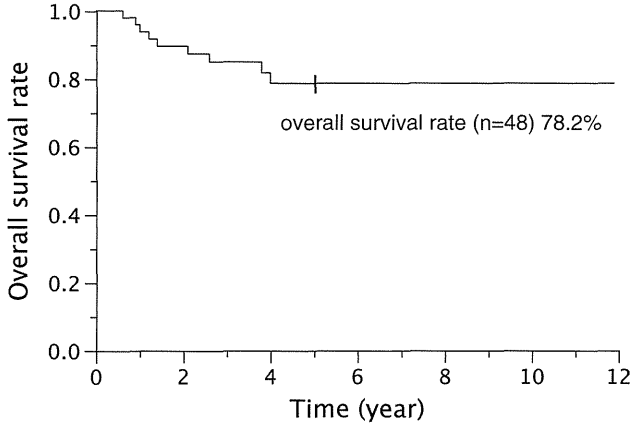

Fig. 1 The overall survival curve of all 48 patients with N0 maxillary sinus cancer after intra-arterial cisplatin infusion with concomitant radiotherapy

arterial administration of cisplatin. Although salvage surgery for persistent primary disease was planned, the presence of late neck recurrence was observed 1 month after the end of initial treatment. Therefore, salvage neck dissection was performed simultaneously with the primary tumor resection.

Although the extent of neck dissection varied, the lymph nodes in the upper and middle jugular chain areas were dissected in all three patients. The patient with metastasis of the intra-parotid lymph node underwent total parotidectomy associated with neck dissection of the upper and middle jugular chain areas. Submandibular nodes (level Ib) were dissected in two patients. The definitive pathological results of the lymph nodes revealed viable tumor cells in all three patients undergoing neck dissection. No post-operative complications were observed in any patient.

Two patients (case 1 and 3) did not undergo any adjunctive treatment after salvage neck dissection. One patient (case 4) underwent adjunctive chemotherapy consisting of oral uracil-tegafur (UFT) for a year after salvage neck dissection.

Discussion

In 1980s, most authors did not advocate ENI for patients with N0 maxillary sinus cancer because of the general belief that nodal metastasis was uncommon in tumors without extensive lymphatic involvement [4–6]. The overall nodal recurrence rate without ENI was reported to be 38 % for squamous cell and undifferentiated carcinomas from the MD Anderson Cancer Center [7]. Based on this finding, researchers at the same institution began to advocate ENI for most patients with carcinomas of the maxillary sinus, with the exception of those with T1 lesions. Following this report, many authors advocated ENI based on the high rate of late neck recurrence and the poor survival of patients with neck recurrence in 1990s–2000s [2, 3]. However, the efficacy of ENI remains controversial due to the fact that there have been few reports indicating the incidence of late neck recurrence in patients with N0 maxillary sinus cancer treated by definitive radiotherapy and chemotherapy without ENI.

ENI has not been used for patients with N0 maxillary sinus cancer when definitive radiotherapy and concomitant superselective intra-arterial chemotherapy were applied in our institution. Our results indicate both a low mortality rate from regional disease (2 %) and a low incidence of late neck recurrence (8.3 %). Based on our uniformed treatment, these results can be considered reliable. The rate of late neck recurrence in our institution was considerably lower than that of recent reports. The reason for the low recurrence rate in our study is speculated to be that intra-

arterially injected cisplatin targeting the primary tumor has a beneficial effect on subclinical metastatic lymph nodes. It has been reported that intra-arterially injected cisplatin can pass, via lymph flow, into the lymph nodes in patients with tongue cancer [8, 9]. These reports appear to support our hypothesis. Further, Jang et al. [10] reported that the incidence of late neck recurrence was 3.3 % in patients with T3-4 N0 maxillary sinus cancer, who underwent definitive radiotherapy without ENI. As 66 % of all participants received induction chemotherapy and 10 % of them received concurrent chemotherapy in this report, it might be that systemic drug application has the capacity to eliminate occult micrometastases in regional lymphatics to a degree, comparable to that of ENI or surgery.

Approaching this subject from a different perspective, Brown et al. investigated the efficacy of elective neck dissection in the management of maxillary sinus cancer. They concluded that elective neck dissection did not contribute to an improved rate of neck control [11]. In terms of surgical treatment, prophylactic intervention for subclinical regional disease was also ineffective.

Patterns of late neck recurrence appear to be varied. It was reported that the most common site of neck recurrence was in level Ib and IIa nodes [2, 12]. Our patients included two with nodal metastasis involving in level IIa, and one involving in level Ib nodes, as well as one patient with an intra-parotid metastatic node. Although nodal metastasis in the intra-parotid area was not common, frontal wall and skin invasion might cause nodal metastasis in this area.

Conclusions

We showed both a low mortality rate from regional disease (2 %) and a low incidence of late neck recurrence (8.3 %) for patients with N0 maxillary sinus cancer, who were treated by RADPLAT. Therefore, it is considered that ENI does not have a significant role in treatment in cases in which definitive radiotherapy and concomitant chemotherapy are applied.

Acknowledgments This study was supported in part by a Health and Labour Sciences Research Grant for Clinical Cancer Research (H22-Gannrinshou-Ippan-017) from the Ministry of Health, Labour and Welfare of Japan, the National Cancer Center Research and Development Fund (23-A-21) of Japan, and a grant-in-aid for Scientific Research (C) (KAKENHI 24592587) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Conflict of interest None declared.

References

1. Grant RN (1970) Cancer statistics 1970. CA Cancer J Clin 8:6–20
2. Paulino AC, Fisher SG, Marks JE (1997) Is prophylactic neck irradiation indicated in patients with squamous cell carcinoma of the maxillary sinus? Int J Radiat Oncol Biol Phys 39:283–289
3. Le QT, Fu KK, Kaplan MJ, Terris DJ, Fee WE, Goffinet DR (2000) Lymph node metastasis in maxillary sinus carcinoma. Int J Radiat Oncol Biol Phys 46:541–549
4. Pezner RD, Moss WT, Tong D, Blasko JC, Griffin TW (1979) Cervical lymph node metastases in patients with squamous cell carcinoma of the maxillary antrum: the role of elective irradiation of the clinically negative neck. Int J Radiat Oncol Biol Phys 5:1977–1980
5. Giri SP, Reddy EK, Gemer LS, Krishnan L, Smalley SR, Evans RG (1992) Management of advanced squamous cell carcinomas of the maxillary sinus. Cancer 69:657–661
6. Lavertu P, Roberts JK, Kraus DH, Levine HL, Wood BG, Medendorp SV, Tucker HM (1989) Squamous cell carcinoma of the paranasal sinuses: the Cleveland Clinic experience 1977–1986. Laryngoscope 99:1130–1136
7. Jiang GL, Ang KK, Peters LJ, Wendt CD, Oswald MJ, Goepfert H (1991) Maxillary sinus carcinomas: natural history and results of postoperative radiotherapy. Radiother Oncol 21:193–200
8. Yokoyama J, Ito S, Ohba S, Fujimaki M, Ikeda K (2011) A novel approach to translymphatic chemotherapy targeting sentinel lymph nodes of patients with oral cancer using intra-arterial chemotherapy—preliminary study. Head Neck Oncol 19(3):42
9. Sakashita T, Homma A, Oridate N, Suzuki S, Hatakeyama H, Kano S, Mizumachi T, Yoshida D, Fujima N, Fukuda S (2012) Platinum concentration in sentinel lymph nodes after preoperative intra-arterial cisplatin chemotherapy targeting primary tongue cancer. Acta Otolaryngol 132:1121–1125
10. Jang NY, Wu HG, Park CI, Heo DS, Kim DW, Lee SH, Rhee CS (2010) Definitive radiotherapy with or without chemotherapy for T3-4N0 squamous cell carcinoma of the maxillary sinus and nasal cavity. Jpn J Clin Oncol 40:542–548
11. Brown JS, Bekiroglu F, Shaw RJ, Woolgar JA, Triantafyllou A, Rogers SN (2013) First report of elective selective neck dissection in the management of squamous cell carcinoma of the maxillary sinus. Br J Oral Maxillofac Surg 51:103–107
12. Yagi K, Fukuda S, Furuta Y, Oridate N, Homma A, Nagahashi T, Inuyama Y (2001) A clinical study on the cervical lymph node metastasis of maxillary sinus carcinoma. Auris Nasus Larynx 28:S77–S81

frontiers in ONCOLOGY

ORIGINAL RESEARCH ARTICLE
published: 23 September 2014
doi: 10.3389/fonc.2014.00261

The potential diagnostic role of the number of ultrasonographic characteristics for patients with thyroid nodules evaluated as Bethesda I–V

Tomohiro Sakashita[1], Akihiro Homma[1]*, Hiromitsu Hatakeyama[1], Takatsugu Mizumachi[1], Satoshi Kano[1], Jun Furusawa[1], Satoshi Iizuka[1], Kimiko Hoshino[1], Kanako C. Hatanaka[2], Koji Oba[3] and Satoshi Fukuda[1]

[1] *Otolaryngology – Head and Neck Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan*
[2] *Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan*
[3] *Translational Research and Clinical Trial Center, Hokkaido University Hospital, Sapporo, Japan*

Edited by:
Makoto Tahara, National Cancer Center Hospital East, Japan

Reviewed by:
Torahiko Nakashima, Kyushu University, Japan
Kenji Okami, Tokai University, Japan

***Correspondence:**
Akihiro Homma, Otolaryngology – Head and Neck Surgery, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan
e-mail: ak-homma@med.hokudai.ac.jp

Objective: Fine-needle aspiration cytology (FNAC) is considered to be the most reliable method of examination for thyroid nodules. However, when thyroid nodules are evaluated as Bethesda I–V, the role of ultrasonography is considered to be enhanced. We investigated the association between a number of ultrasonographic (US) characteristics and the risk of thyroid malignancy, and assessed the optimal compromise on the number of US characteristics for predicting thyroid malignancy.

Methods: Seventy-three patients, whose thyroid nodules were evaluated as Bethesda I–V by FNAC prior to surgery, were treated surgically. A number of US characteristics, such as microcalcification, irregular margins, hypoechogenicity, a taller-than-wide shape, and the absence of halo sign, were assessed before surgery. The optimal compromise on the number of US characteristics was analyzed using a receiver operating characteristics (ROC) curve. The area under the ROC curve (AUC) represents the overall discriminatory ability of a test.

Results: The risk of malignancy was 11.8% in patients without any US characteristics, 44.4% in those with one characteristic, 61.5% in those with two characteristics, 75% in those with three characteristics, 90% in those with four characteristics, and 100% in those with five characteristics. The AUC was favorable (0.81599). At least two US characteristics were revealed to be the optimal compromise on the number of US characteristics based on the ROC curve.

Conclusion: We proved the role of the number of US characteristics in predicting thyroid malignancy. It was thought that a surgical approach should be considered for patients with at least two US characteristics.

Keywords: thyroid cancer, thyroidectomy, fine-needle aspiration cytology, ultrasound image, ROC curve

INTRODUCTION

Fine-needle aspiration cytology (FNAC) is considered as the most reliable method of examination by which to make decisions regarding surgical treatment for patients with thyroid nodules (1, 2). To standardize FNAC reports, the National Cancer Institute (NCI) hosted an "NCI thyroid fine-needle aspiration state of the science conference," which led to the formation of "The Bethesda system for reporting thyroid cytopathology" (3–5). On the other hand, ultrasonography has been reported to be effective in predicting thyroid malignancies (6, 7). We believe that the role of ultrasonography is enhanced for patients with nodules that were not diagnosed definitively as malignant by FNAC. We assessed the accuracy of each ultrasonographic (US) characteristic for patients with thyroid nodules classified as Bethesda I–V. We also focused on the number of US characteristics, and determined the optimal compromise on the number of US characteristics for predicting thyroid malignancies.

MATERIALS AND METHODS

PATIENTS

A total of 185 patients with thyroid nodule were surgically treated between July 2010 and December 2013 in the Department of Otolaryngology-Head and Neck Surgery, Hokkaido University, Sapporo, Japan. Of these, 73 patients, whose thyroid nodules were evaluated as Bethesda I–V by FNAC before surgery, were eligible for this study. These patients consisted of 52 women and 21 men, with a median age of 58 years old (range, 24–77 years). Approval for this study was obtained from the institutional review board of Hokkaido University.

PREOPERATIVE EVALUATION AND ULTRASONOGRAPHIC DIAGNOSTIC CRITERIA

Certified head and neck surgeons performed ultrasonography for patients with thyroid nodules before surgery using a HI VISION Ascendus system (HITACHI ALOKA Medical, Tokyo, Japan).

Table 1 | The modified ultrasonographic diagnostic criteria.

Microcalcification Irregular margins Hypoechogenicity Taller-than-wide shape Absence of halo sign	Nodules defined as positive if any one of these characteristics were observed

Thyroid nodules were evaluated by B mode, and nodule size was recorded. US characteristics, such as microcalcification, irregular margins, hypoechogenicity, taller-than-wide shape, and the absence of halo sign, were assessed according to previous reports (6, 7). The US diagnostic criteria defined nodules as positive if any one of these five characteristics were observed (**Table 1**). FNAC was performed before surgery for all patients with a 21-gage needle attached to a 50 cc disposable syringe using US guidance. Two alcohol-fixed smears were prepared for Papanicolaou staining. On-site evaluation was not performed routinely. FNAC was evaluated by pathologists in accordance with the Bethesda system (3–5).

SURGICAL TREATMENT AND POSTOPERATIVE EVALUATION

Lobectomy or total thyroidectomy was performed based on the result of FNAC, the extent of nodules, the nodal status, or the patient's wishes. Paratracheal nodal dissection or lateral neck dissection was added according to the nodal status. The removed thyroid nodules were assessed pathologically.

STATISTICAL ANALYSIS

We calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each US characteristic. The risk of malignancy was also calculated according to the number of US characteristics. The optimal compromise on the number of US characteristics was analyzed using a receiver operating characteristics (ROC) curve. The ROC curve plots sensitivity against (one-specificity) for all possible thresholds in a binary classification task. The area under the ROC curve (AUC) represents the overall discriminatory ability of a test, where a value of 1.0 denotes perfect ability and a value of 0.5 denotes no ability.

RESULTS

THE BETHESDA CLASSIFICATION

Thyroid nodules were evaluated as non-diagnostic or unsatisfactory (Bethesda I) in 10 patients, benign (Bethesda II) in 22 patients, atypia of undetermined significance or follicular lesion of undetermined significance (Bethesda III) in 12 patients, follicular neoplasm, or suspicious for a follicular neoplasm (Bethesda IV) in 9 patients, and suspicious for malignancy (Bethesda V) in 20 patients by FNAC.

SURGERY AND FINAL PATHOLOGICAL RESULTS

The reason for surgery was suspicious for malignancy by FNAC in 20 patients, the presence of US abnormalities in 35 patients, large size (>3 cm) in 12 patients, and the patient's wish for a diagnostic lobectomy instead of repeat FNAC in 6 patients.

Based on final pathological results, benign lesions were observed in 34 patients, consisting of 24 cases of adenomatous goiter, 9 of follicular adenoma, and 1 of benign cyst. Malignant tumors were observed in 39 patients, consisting of one case of anaplastic carcinoma, one of poorly differentiated carcinoma, 34 of differentiated papillary carcinoma, and 3 of minimally invasive follicular carcinoma. **Table 2** shows the correlation between Bethesda classification and final pathological diagnosis.

Table 2 | The risk of malignancy according to Bethesda classification.

Bethesda classification	No. of patients	Final pathology		The risk of malignancy (%)
		Malignant	Benign	
I	10	6	4	60.0
II	22	8	14	36.4
III	12	5	7	41.7
IV	9	2	7	22.2
V	20	18	2	90.0

Table 3 | The correlation between final pathology and evaluation of the ultrasonographic (US) criteria (sensitivity 94.9%, specificity 44.1%, positive predictive value 66.1%, negative predictive value 88.2%).

	Final pathology		Total
	Malignant	Benign	
US Criteria			
Positive	37	19	56
Negative	2	15	17
Total	39	34	73

US CHARACTERISTICS AND DIAGNOSTIC VALUE

The median maximum nodule diameter was 22 mm (range, 5–70 mm). Microcalcification was observed in 30 patients, irregular margins in 23 patients, hypoechogenicity in 24 patients, taller-than-wide shape in 14 patients, and an absence of halo sign in 44 patients.

With the use of the modified US diagnostic criteria, 56 patients were classified as positive. In 37 of these 56 patients, malignant thyroid diseases were observed. When using diagnostic criteria, the sensitivity, specificity, PPV, and NPV were calculated as 94.9, 44.1, 66.1, and 88.2%, respectively (**Table 3**).

Table 4 shows the sensitivity, specificity, PPV, and NPV for each US characteristic and tumor size.

THE NUMBER OF US CHARACTERISTICS AND ROC CURVE ANALYSIS

Table 5 shows the risk of malignancy according to the number of US characteristics. Sensitivity and specificity were indicated for each number of US characteristics in **Table 6**. The analysis of the association between the risk of malignancy and the number of US characteristics is shown in **Figure 1** with an ROC curve showing the plots of all thresholds. The value of AUC was 0.81599. At least two US characteristics was revealed to be the optimal compromise on the number of US characteristics based on the ROC curve.

Table 4 | Diagnostic value of each ultrasonographic characteristic.

Characteristics	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Tumor size >3 cm	23.1	61.8	40.9	41.2
Microcalcification	59.0	79.4	76.7	62.8
Irregular margins	51.3	91.2	87.0	62.0
Hypoechogenicity	46.2	82.4	75.0	57.1
Taller-than-wide shape	30.8	94.1	85.7	54.2
Absence of halo sign	74.4	55.9	65.9	65.5

PPV, positive predictive value, NPV, negative predictive value.

Table 5 | The risk of malignancy according to the number of ultrasonographic (US) characteristics.

The number of US characteristics	No. of patients	Final pathology		The risk of malignancy (%)
		Malignant	Benign	
5	3	3	0	100.0
4	10	9	1	90.0
3	12	9	3	75.0
2	13	8	5	61.5
1	18	8	10	44.4
0	17	2	15	11.8

Table 6 | The sensitivity and specificity of each number of ultrasonographic (US) characteristics for predicting thyroid malignancies.

The number of US characteristics	Sensitivity (%)	Specificity (%)
5	7.7	100.0
4	30.8	97.1
3	53.9	88.2
2	74.4	73.5
1	94.9	44.1
0	100.0	0.0

ANALYSIS OF 34 CASES DIAGNOSED WITH DIFFERENTIATED PAPILLARY CARCINOMA

Of 34 cases diagnosed with differentiated papillary carcinomas, five cases were evaluated as Bethesda I, seven cases were evaluated as Bethesda II, three cases were evaluated as Bethesda III, two cases were evaluated as Bethesda IV, and 17 cases were evaluated as Bethesda V. Of these 34, 3 patients had 5 US characteristics, 7 patients had 4 US characteristics, 9 patients had 3 US characteristics, 8 patients had 2 US characteristics, 6 patients had 1 US characteristic, and 1 patient had no US characteristics.

DISCUSSION

Both accuracy and value of FNAC for the preoperative evaluation of thyroid nodules have been established. When the cytological diagnosis is malignant, the PPV is >99%, and when it is benign, the false negative is typically <5% (4, 8). On the other hand, many

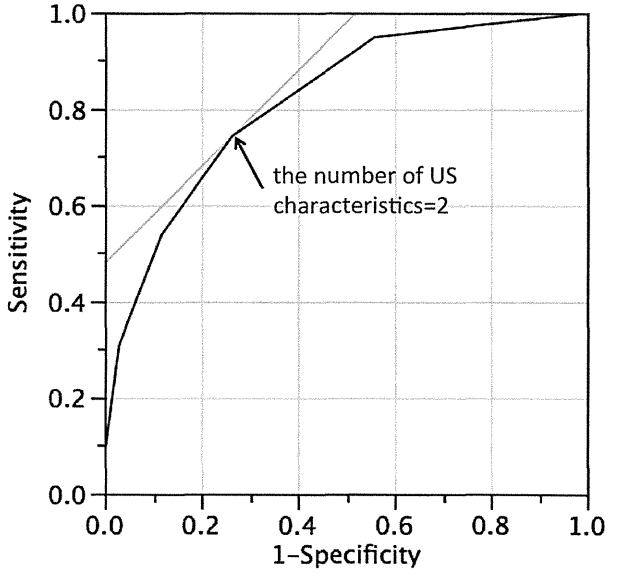

FIGURE 1 | The receiver operating characteristics (ROC) curve showing the predictive value for thyroid malignancy based on the number of ultrasonographic (US) characteristics. The area under the ROC curve (AUC) was 0.81599.

authors have also found ultrasonography to be effective in predicting thyroid malignancies. Kim et al. reported the effectiveness of the US diagnostic criteria, which was defined as positive if any one of the US characteristics, such as microcalcification, irregular margins, hypoechogenicity, or taller-than-wide shape, was observed (6). They applied these criteria to patients with thyroid nodules and found that the sensitivity, specificity, PPV, and NPV were 94, 66, 56, and 96%, respectively. Rago et al. also reported that of the absence of halo sign was useful in predicting thyroid malignancies (7). In current study, we added this feature to the US criteria reported by Kim et al., and evaluated these modified US diagnostic criteria on patients with Bethesda I–V. We found that these criteria had almost the same level of accuracy as that reported by Kim et al. Although we did not find any evidence that a specific feature was particularly effective in predicting thyroid malignancies, both irregular margins and taller-than-wide shape had high PPVs (87 and 85.7%, respectively), and might be the most predictive characteristics. In addition, we were unable to prove the efficacy of nodule size in predicting thyroid malignancies. From the results of our study, we believe that nodule size should not be included in the US diagnostic criteria. The NPV of our criteria (88.2%) was satisfactory. Therefore, it might be acceptable to recommend repeat FNAC for patients without any US characteristics.

Smith-Bindman et al. suggested classifying patients with thyroid nodules according to the number of US characteristics, such as nodule size (>2 cm), microcalcification, or solid nodules (9). It was suggested that patients with <2 US characteristics have a risk of malignancy of 5 per 1000 patients, so that it was considered acceptable for biopsy or diagnostic lobectomy to be deferred in such patients. From the current study, our ROC curve analysis indicated that at least two US characteristics were optimal compromise, and we proved that the number of US characteristics

was correlated significantly with the risk of malignancy, as the AUC of the ROC curve showed a favorable value (0.81599). This indicated that patients with at least two US characteristics had a risk of malignancy of 76%. Although repeat FNAC is convenient and minimally invasive, we believe that a surgical approach should be considered for patients with at least two US characteristics.

Marchevsky et al. reviewed the risk of malignancies predicted by FNAC. The rates of malignancies detected on thyroidectomy were 75% in patients with Bethesda I, 32.2% in those with Bethesda II, 37.9% in those with Bethesda III, 27.3% in those with Bethesda IV, and 100% in those with Bethesda V (10). Although our results were comparable to previous reports, it is regarded inevitable that the risk of malignancy by FNAC classification should vary quite markedly among institutions.

Ohori et al. reviewed the risk of malignancy in patients with Bethesda III, and found that the risk ratio ranged from 6 to 48% (11). Gweon et al. also reported the risk of malignancy based on thyroidectomy and/or FNAC was 70% in patients with Bethesda III. They found that the adoption of US evaluation elevated the accuracy of the diagnosis of malignancies to 85–100% (12). From these reports, it appears to be acceptable to make the decision to perform surgery on the basis of US findings for patients with thyroid nodules categorized as Bethesda III.

The limitations of this study include its retrospective nature, and the limited study population. Our study was designed to determine how to reduce unnecessary and excessive thyroid surveillance and lobectomy. Therefore, we limited inclusion eligibility to those patients undergoing surgery and with Bethesda I–V nodules. Our data might not, therefore, be applicable to every case with Bethesda I–V nodules. In addition, the accuracy of the FNAC was very low in our study. In previous reports, patients with thyroid nodules evaluated as Bethesda I–III were recommended for repeat FNAC or clinical follow-up (4, 5). However, 44 patients with nodules evaluated as Bethesda I–III underwent thyroidectomy in our study. If we undertook repeat FNAC for these 44 patients, the accuracy of the FNAC would be improved. However, we would like to recommend that the decision to undertake thyroidectomy be based on the number of US characteristics instead of repeat FNAC for patients with thyroid nodules evaluated as Bethesda I–III.

We focused on 34 cases pathologically diagnosed with differentiated papillary carcinoma. Of these 34, 12 were false negative based on FNAC findings, including 7 with Bethesda II-benign, 3 with Bethesda III-atypia of undetermined significance or follicular lesion of undetermined significance, and 2 with Bethesda IV-follicular neoplasm. After excluding five cases with Bethesda I-unsatisfactory, the false negative rate for FNAC was calculated as 41.4% (12/29). Using the criteria of at least two US characteristics, false negative findings based on the number of US characteristics were observed in seven patients (20.6%, 7/34). This focus on differentiated papillary carcinomas reconfirmed the beneficial role of the number of US characteristics due to the acceptable false negative rate.

In conclusion, we applied a modified set of US criteria, and proved the efficacy of the number of US characteristics in predicting thyroid malignancies. We believe that a surgical approach should be considered for patients with at least two US characteristics.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Young Scientists (B) Number 26861352 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. Revised American thyroid association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* (2009) **19**:1167–214. doi:10.1089/thy.2009.0110
2. Gharib H, Goellner JR. Fine-needle aspiration biopsy of the thyroid: an appraisal. *Ann Intern Med* (1993) **15**(118):282–9. doi:10.7326/0003-4819-118-4-199302150-00007
3. Baloch ZW, LiVolsi VA, Asa SL, Rosai J, Merino MJ, Randolph G, et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. *Diagn Cytopathol* (2008) **36**:425–37. doi:10.1002/dc.20830
4. Cibas ES, Ali SZ. The Bethesda system for reporting thyroid cytopathology. *Thyroid* (2009) **19**:1159–65. doi:10.1089/thy.2009.0274
5. Ali SZ. Thyroid cytopathology: Bethesda and beyond. *Acta Cytol* (2011) **55**:4–12. doi:10.1159/000322365
6. Kim EK, Park CS, Chung WY, Oh KK, Kim DI, Lee JT, et al. New sonographic criteria for recommending fine-needle aspiration biopsy of nonpalpable solid nodules of the thyroid. *AJR Am J Roentgenol* (2002) **178**:687–91. doi:10.2214/ajr.178.3.1780687
7. Rago T, Vitti P, Chiovato L, Mazzeo S, De Liperi A, Miccoli P, et al. Role of conventional ultrasonography and color flow-Doppler sonography in predicting malignancy in 'cold' thyroid nodules. *Eur J Endocrinol* (1998) **138**:41–6. doi:10.1530/eje.0.1380041
8. Olson MT, Clark DP, Erozan YS, Ali SZ. Spectrum of risk of malignancy in subcategories of 'atypia of undetermined significance'. *Acta Cytol* (2011) **55**:518–25. doi:10.1159/000333232
9. Smith-Bindman R, Lebda P, Feldstein VA, Sellami D, Goldstein RB, Brasic N, et al. Risk of thyroid cancer based on thyroid ultrasound imaging characteristics: results of a population-based study. *JAMA Intern Med* (2013) **173**:1788–96. doi:10.1001/jamainternmed.2013.9245
10. Marchevsky AM, Walts AE, Bose S, Gupta R, Fan X, Frishberg D, et al. Evidence-based evaluation of the risks of malignancy predicted by thyroid fine-needle aspiration biopsies. *Diagn Cytopathol* (2010) **38**:252–9. doi:10.1002/dc.21185
11. Ohori NP, Schoedel KE. Variability in the atypia of undetermined significance/follicular lesion of undetermined significance diagnosis in the Bethesda system for reporting thyroid cytopathology: sources and recommendations. *Acta Cytol* (2011) **55**:492–8. doi:10.1159/000334218
12. Gweon HM, Son EJ, Youk JH, Kim JA. Thyroid nodules with Bethesda system III cytology: can ultrasonography guide the next step? *Ann Surg Oncol* (2013) **20**:3083–8. doi:10.1245/s10434-013-2990-x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 August 2014; accepted: 07 September 2014; published online: 23 September 2014.

Citation: Sakashita T, Homma A, Hatakeyama H, Mizumachi T, Kano S, Furusawa J, Iizuka S, Hoshino K, Hatanaka KC, Oba K and Fukuda S (2014) The potential diagnostic role of the number of ultrasonographic characteristics for patients with thyroid nodules evaluated as Bethesda I–V. Front. Oncol. 4:261. doi: 10.3389/fonc.2014.00261

This article was submitted to Head and Neck Cancer, a section of the journal Frontiers in Oncology.

PLOS ONE

Effects of Aprepitant on the Pharmacokinetics of Controlled-Release Oral Oxycodone in Cancer Patients

Yutaka Fujiwara[1,3]*, Masanori Toyoda[1], Naoko Chayahara[1], Naomi Kiyota[1], Takanobu Shimada[1], Yoshinori Imamura[1], Toru Mukohara[1,2], Hironobu Minami[1,2]

1 Division of Medical Oncology/Hematology, Kobe University Graduate School of Medicine, Kobe, Japan, **2** Cancer Center, Kobe University Graduate School of Medicine, Kobe, Japan, **3** Division of Investigational Cancer Therapeutics, Exploratory Oncology Research & Clinical Trial Center, National Cancer Center Hospital, Chiba, Japan

Abstract

Purpose: Oxycodone is a μ-opioid receptor agonist widely used in the treatment of cancer pain. The predominant metabolic pathway of oxycodone is CYP3A4-mediated N-demethylation to noroxycodone, while a minor proportion undergoes 3-O-demethylation to oxymorphone by CYP2D6. The aim of this study was to investigate the effects of the mild CYP3A4 inhibitor aprepitant on the pharmacokinetics of orally administered controlled-release (CR) oxycodone.

Method: This study design was an open-label, single-sequence with two phases in cancer patients with pain who continued to be administered orally with multiple doses of CR oxycodone every 8 or 12 hours. Plasma concentration of oxycodone and its metabolites were measured up to 8 hours after administration as follows: on day 1, CR oxycodone was administered alone; on day 2, CR oxycodone was administered with aprepitant (125 mg, at the same time of oxycodone dosing in the morning). The steady-state trough concentrations (Css) were measured from day 1 to day 3.

Results: Aprepitant increased the area under the plasma concentration-time curve (AUC_{0-8}) of oxycodone by 25% ($p<0.001$) and of oxymorphone by 34% ($p<0.001$), as well as decreased the AUC_{0-8} of noroxycodone by 14% ($p<0.001$). Moreover, aprepitant increased Css of oxycodone by 57% ($p=0.001$) and of oxymorphone by 36% ($p<0.001$) and decreased Css of noroxycodone by 24% ($p=0.02$) at day 3 compared to day 1.

Conclusions: The clinical use of aprepitant in patients receiving multiple doses of CR oxycodone for cancer pain significantly altered plasma concentration levels, but would not appear to need modification of the CR oxycodone dose.

Trial Registration: UMIN.ac.jp UMIN000003580.

Citation: Fujiwara Y, Toyoda M, Chayahara N, Kiyota N, Shimada T, et al. (2014) Effects of Aprepitant on the Pharmacokinetics of Controlled-Release Oral Oxycodone in Cancer Patients. PLoS ONE 9(8): e104215. doi:10.1371/journal.pone.0104215

Editor: Emanuel F. Petricoin, George Mason University, United States of America

Received March 21, 2014; **Accepted** July 1, 2014; **Published** August 14, 2014

Funding: This study was supported by a grant for Research on Applying Health Technology from the Ministry of Health, Labour, and Welfare of Japan and Yokoyama-Rinsyo foundation. However, the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: yutakafu@ncc.go.jp

Introduction

Oxycodone is a μ-opioid receptor agonist which is widely used in the treatment of cancer pain and chronic pain [1]. It is a semi-synthetic form of morphine with similar analgesic properties and side effects such as nausea, vomiting, constipation, somnolence, dizziness and pruritus [2]. At high dose or overdoses, oxycodone can cause shallow respiratory depression, somnolence progressing to stupor or coma, skeletal muscle flaccidity, etc. The oral bioavailability of oxycodone is 60 to 87%, and is higher than that of morphine [3–5]. Only 10% of the oxycodone dose is excreted unchanged in the urine and it is extensively metabolized by duodenal and hepatic cytochrome P450 (CYP) isozymes [6] [7]. The predominant metabolic pathway of oxycodone is CYP3A4-mediated N-demethylation to noroxycodone, while a minor proportion undergoes 3-O-demethylation CYP2D6 to oxymorphone, which is the active metabolite. Further oxidation of these metabolites via CYP2D6 (and CYP3A4) yields noroxymorphine [6]. Both of these metabolites are further metabolized into noroxymorphine.

Aprepitant, an orally available, selective neurokinin-1 receptor agonist, is effective for both acute and delayed chemotherapy-induced nausea and vomiting (CINV) and is used in combination with a 5-hydroxytryptamine-3 ($5HT_3$) antagonist and a corticosteroid (e.g., dexamethasone) for the treatment of moderately and highly emetogenic chemotherapy. The recommended dose of aprepitant is 125 mg prior to chemotherapy on day 1 and 80 mg once daily on days 2 and 3 (125-mg/80-mg regimen).

Aprepitant is metabolized by CYP isozymes 1A2, 2C19, and 3A4, and was shown to be a moderate inhibitor of CYP3A4 (K_i of about 10 μM for 1′ and 4-hydroxylation of midazolam and N-demethylation of diltiazem, respectively) in vitro and a very weak inhibitor of CYP2C19 and CYP2C9 [8]. Moreover, drug-drug interaction studies have indicated that aprepitant can inhibit CYP3A4 enzyme activity. When the standard oral dexamethasone regimen for CINV (20 mg on day 1 and 8 mg on days 2 to 5) was

given concomitantly with aprepitant, the dexamethasone area under the time-concentration curve (AUC) from 0 to 24 hours increased approximately 2-fold on both day 1 and day 5 compared with the standard oral dexamethasone regimen alone [9]. When the methylprednisolone regimen consisted of 125 mg intravenously on day 1 and 40 mg orally on days 2 to 3, aprepitant increased the AUC of intravenous methylprednisolone 1.3-fold on day 1 and of oral methylprednisolone 2.5-fold on day 3 [9]. Conversely, several studies have not demonstrated that aprepitant use mediated clinically relevant effects on the pharmacokinetics of intravenously administered docetaxel or vinorelbine [10] [11].

At the 125-mg/80-mg regimen used for oral aprepitant administration for CINV, the peak plasma concentrations (Cmax) of 1,600 ng/mL (around 3.0 μM) and 1,400 ng/mL (around 2.6 μM) were reached in approximately 4 hours (Tmax) on day 1 and day 3, respectively [12]. As the intestinal drug concentration following oral administration is even higher than the plasma concentration, it is expected that orally-administered aprepitant inhibits intestinal CYP3A4 greater than intravenously-administered aprepitant and that orally co-administered drug is affected to a greater extent by the inhibitory effect of intestinal CYP3A4 than intravenously co-administered drug [9,13].

Concomitant use of oxycodone and aprepitant is used in clinical practice for cancer patient care. However, aprepitant might have the potential to increase the plasma concentrations of oxycodone and its metabolites via inhibition of CYP3A-mediated metabolism of oxycodone. As a result, the side effects of oxycodone may increase. In this study, we have therefore investigated the possible effects of the mild CYP3A4 inhibitor aprepitant on the pharmacokinetics of orally administered CR oxycodone in patients with cancer pain.

Methods

The protocol for this trial and supporting TREND checklist are available as supporting information; see Checklist S1, Protocol S1 and S2.

Patient selection criteria

The subjects were enrolled in patients whom continued to be administered CR oxycodone twice or three times daily for cancer pain and were planned to receive chemotherapy with aprepitant for CINV. Within the last 3 or more days to reach steady state, the subjects had received a fixed dose of CR oxycodone. Additional eligibility criteria were age≥18 years, histologically confirmed malignant solid tumor, and adequate organ function [serum total bilirubin less than 1.5×upper limits of normal (ULN), aspartate aminotransferase (AST) less than 2.5×ULN, alanine aminotransferase (ALT) less than 2.5×ULN, and serum creatinine less than 1.5×ULN]. Patients were excluded if they had gastrointestinal disorders that could affect ingestion or absorption of either CR oxycodone or aprepitant, and if they were receiving or likely to receive drugs or food that could act as potent CYP3A4 or CYP2D6 inhibitors or inducers. All patients provided written informed consent and study approval was obtained from the Institutional Review Board of Kobe University Hospital.

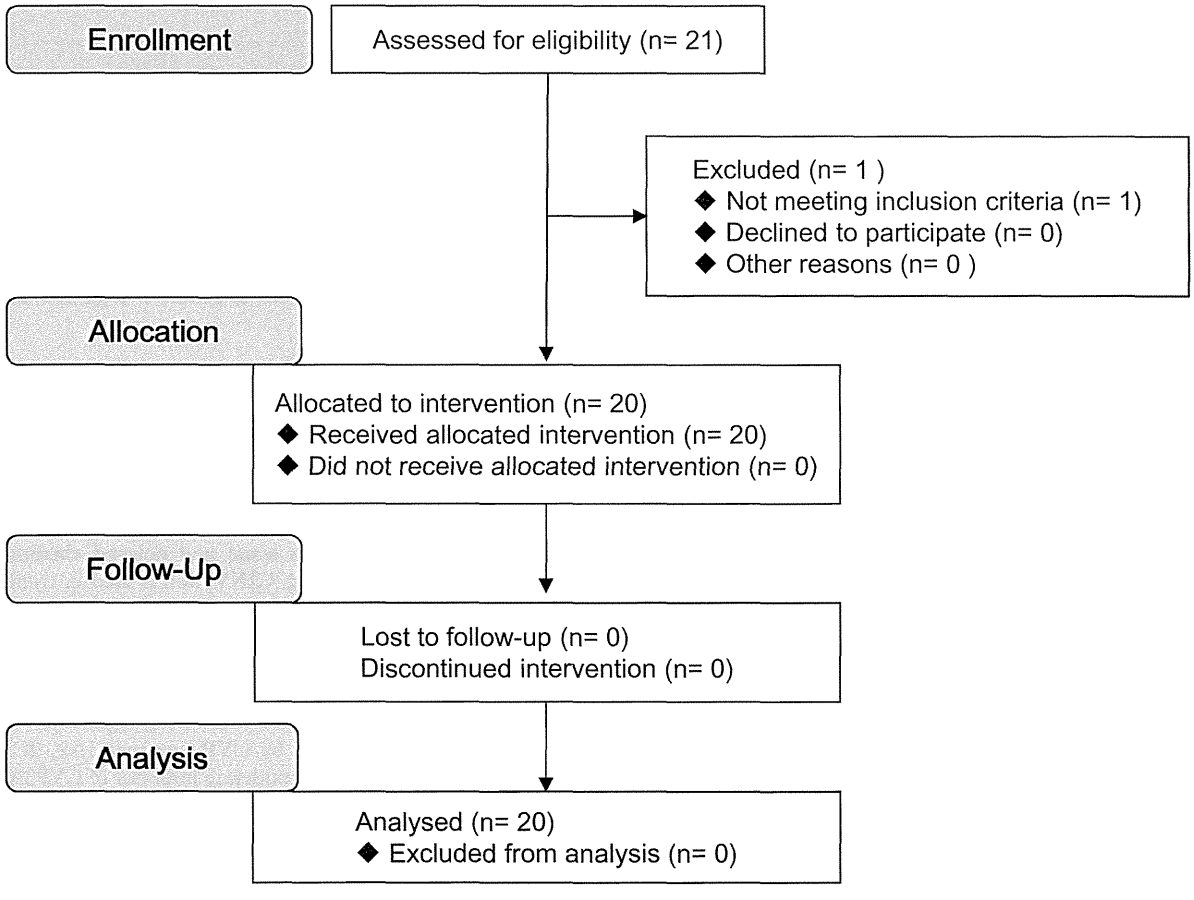

Figure 1. CONSORT flow diagram.
doi:10.1371/journal.pone.0104215.g001

Table 1. Patient characteristics.

		Number of Patients (n = 20)
Gender	Male/female	17 (85%)/3 (15%)
Age	Median (range)	66.5 (44–77)
ECOG PS	1/2	13 (65%)/7 (35%)
Height (cm)	Median (range)	164.4 (138.5–177.1)
Weight (kg)	Median (range)	59.6 (37–77)
BSA (m^2)	Median (range)	1.64 (1.19–1.90)
Cancer type	Pancreatic cancer	8 (40%)
	Head and Neck cancer	4 (20%)
	NSCLC	2 (10%)
	CRC	2 (10%)
	CUP	2 (10%)
	Endometrial cancer	1 (5%)
	Cholangiocarcinoma	1 (5%)
Clinical stage	IV	20 (100%)
Anti-cancer agent	Platinum agent	8 (40%)
	Gemcitabine	7 (35%)
	Fluoropyrimidine	5 (25%)
	Taxanes	4 (20%)
	Anthracyclines	2 (10%)
	Irinotecan	2 (10%)
	Sunitinib	1 (5%)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; NSCLC, non-small cell lung cancer; CRC, colorectal cancer; CUP, cancer of unknown primary.

doi:10.1371/journal.pone.0104215.t001

Study design

This study which was an open-label, two-period, single-sequence design was conducted at Kobe University Hospital. Patients were administered regularly with multiple-doses of oral CR oxycodone every 8 or 12 hours. Each patient was administered with the appropriate dose of oral CR oxycodone for their cancer pain. They received CR oxycodone alone (period A) on the previous day of planned chemotherapy and CR oxycodone with aprepitant (period B) on the day of chemotherapy. On the morning of period B, aprepitant was taken orally at the same time as CR oxycodone more than one hour prior to chemotherapy. Patients were participated in this study during blood sampling. Patients in hospital were given the dose of anticancer agents according to standard treatment schedule for their tumor types and were allowed to receive an antiemetic treatment with dexamethasone and a $5HT_3$ receptor antagonist where appropriate.

Outcome

The study objective was to investigate aprepitant might have the potential to increase the plasma concentrations of oxycodone and its metabolites via inhibition of CYP3A-mediated metabolism of oxycodone. The primary endpoint was pharmacokinetics of oxycodone and its metabolites with and without aprepitant administration. Secondary endpoints were safety and adverse event including nausea, vomiting, constipation, and somnolence. Patient characteristics and medication information were recorded

Table 2. Dose of Oxycodone.

Dose	Frequency of administration	Daily dosage	Number of patients
5 mg	every 12 hours	10 mg	6 (30%)
	every 8 hours	15 mg	2 (10%)
10 mg	every 12 hours	20 mg	6 (30%)
	every 8 hours	30 mg	3 (15%)
15 mg	every 12 hours	30 mg	1 (5%)
20 mg	every 12 hours	40 mg	1 (5%)
	every 8 hours	60 mg	1 (5%)

doi:10.1371/journal.pone.0104215.t002

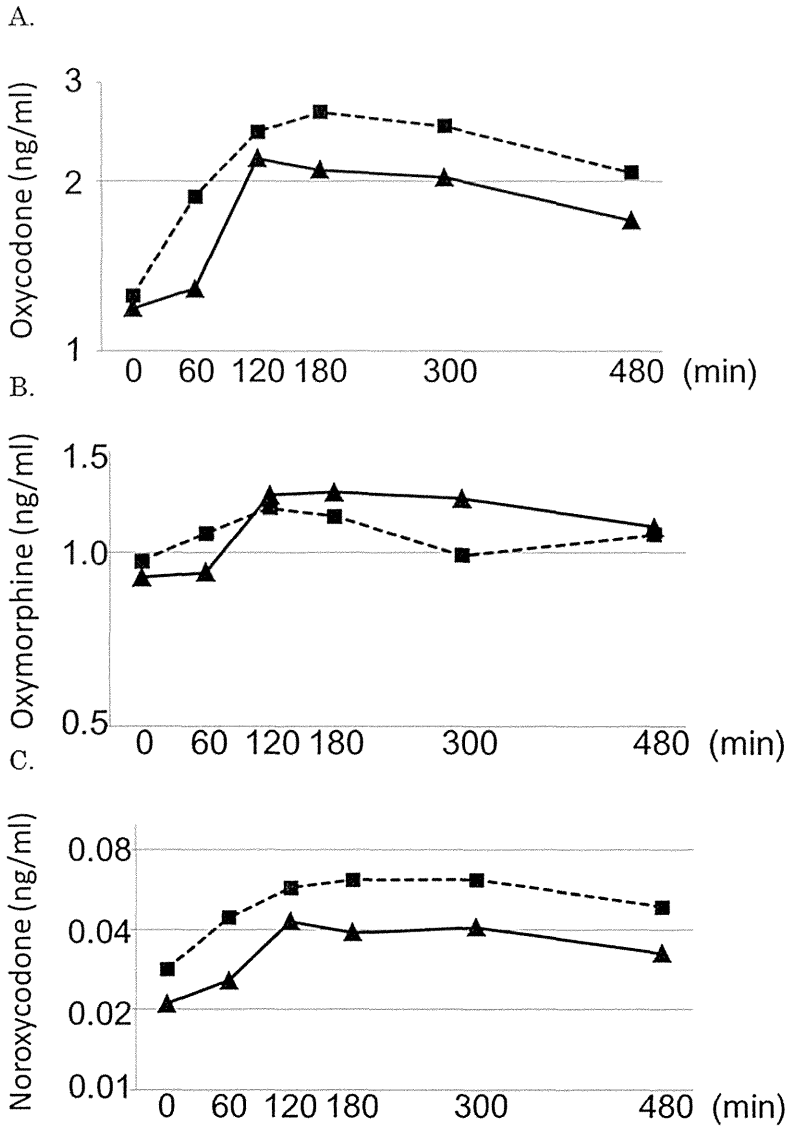

Figure 2. Mean plasma concentration curves of oxycodone, noroxycodone, and oxymorphone in patients (n=6) who were administered with 10 mg of CR oxycodone every 12 hours alone (period A, triangles) or with aprepitant (period B, squares). ▲ without aprepitant, ■ with aprepitant.
doi:10.1371/journal.pone.0104215.g002

throughout the study. Adverse events were evaluated using the CTCAE v4.0.

Blood sampling

Blood samples for pharmacokinetic analysis were collected immediately before and 1, 2, 3, 5, and 8 hour after administration of oxycodone in periods A and B. An additional sample was collected to allow for analysis of trough concentration before administration of oxycodone in the morning on the following day of period B. After blood was collected in lithium heparin-containing tubes, plasma was separated within 30 min by centrifugation at 1,500×g for 10 min at 4°C and stored at −80°C until analysis. Plasma concentrations of oxycodone, noroxycodone, and oxymorphone were determined using a liquid chromatography tandem mass spectrometric method. The lower limit of quantification was 0.1 ng/ml.

Pharmacokinetic analysis

Pharmacokinetic variables of oxycodone, noroxycodone, and oxymorphone were determined using the Pheoenix WinNonlin pharmacokinetic program (Pharsight, Mountain View, California). The Cmax and time to maximum concentration (Tmax) were observed directly from the data. The AUC with extrapolation to 8 hour (AUC_{0-8}) was calculated by the trapezoidal rule. The linear trapezoidal rule was used for successive increasing concentration values, and the logarithmic trapezoidal rule for decreasing concentration values. Metabolite-to-parent drug AUC ratios (AUCm/AUCp) were calculated to compare the relative abundance of each metabolite.

Statistical Analysis

This study was designed in order to exclude a clinically significant higher exposure to oxycodone and its metabolites. The null hypothesis was that coadministration of aprepitant would not

Table 3. Pharmacokinetic parameter of oxycodone and its metabolites.

	Oxycodone			Noroxycodone	Oxymorphone
	Cmax (ng/mL)	Tmax (hr)	$AUC_{0\rightarrow8}$ (ng*hr/mL)	$AUC_{0\rightarrow8}$ (ng*hr/mL)	$AUC_{0\rightarrow8}$ (ng*hr/mL)
Number of patients	20	20	20	20	15**
Without aprepitant	2.28	2.67	882	718	14.9
	(31.4%)	(57.7%)	(35.7%)	(45.2%)	(78.0%)
With aprepitant	2.79	3.62	1102	616	20.7
	(28.0%)	(32.1%)	(29.9%)	(51.6%)	(65.8%)
ratio	1.22		1.25	0.86	1.34
	(1.11–1.34)		(1.14–1.36)	(0.81–0.91)	(1.20–1.49)
p-value*	0.0002	0.07	0.00004	0.00005	0.00004

Abbreviations: Cmax, peak plasma concentration; Tmax, time to peak plasma concentration; $AUC_{0\rightarrow8}$, area under the time-concentration curve from 0 to 8 hours; ratio, the ratio of the geometric mean value of CR oxycodone with aprepitant to those without aprepitant.
Geometric mean (% coefficient variance).
Values were corrected for dose, assuming that all patients received 20 mg of oxycodone.
*Paired t-test for difference between logarithmic geometric means (two-sided).
**Five patients were excluded due to below lower limit of quantitation.
doi:10.1371/journal.pone.0104215.t003

increase the plasma concentration of oxycodone to a clinically meaningful degree, i.e., the ratio of the geometric mean $AUC_{0\rightarrow8}$ for oxycodone between period A and period B would be <1.33. Package insert of oxycodone reports that the AUC of oxycodone in steady state was 216.2 ± 97.4 ng.hr/ml [mean $\pm$ standard deviation, coefficient of variance (CV) was 45.1%] in patients with cancer pain ($n = 32$). We estimated that 20 subjects were needed to detect a 33% difference in the $AUC_{0\rightarrow8}$ for oxycodone at a power of 80% and level of significance $p<0.05$ (two-sided). The calculations used the sample size procedures in NCSS PASS 11 software. Data are expressed as the geometric mean $\pm$ SD. Statistical significance of logarithmic geometric means in AUC and Cmax was analyzed using a paired Student's t-test, with a probability level of 0.05 used as the criterion of significance. Tmax was analyzed using a Wilcoxon signed-rank test. All statistical analyses were performed with NCSS 2007 (NCSS, LLC. Kaysville, UT).

Results

Patient population

Twenty one patients were assessed for eligibility and 20 patients were allocated to intervention from September 2010 to December 2012 (Figure 1). Their characteristics are listed in Table 1. There were 17 men and 3 women with Eastern Cooperative Oncology Group performance status 1 to 2. The predominant tumor types were pancreatic cancer and head and neck cancer, with all patients having stage IV disease. Each patient was administered regularly with the appropriate dose of oral CR oxycodone every 8

Table 4. Trough concentrations of oxycodone and its metabolites.

	Oxycodone		Noroxycodone		Oxymorphone	
	N	(ng/mL)	N	(ng/mL)	N	(ng/mL)
Day 1 pre-dose	20	1.29	20	1.28	14	0.0243
Without aprepitant		(53.1%)		(46.2%)		(72.7%)
Day 2 pre-dose	20	1.22	20	1.23	14	0.0277
Without aprepitant		(49.3%)		(47.8%)		(68.8%)
Day 3 pre-dose	19	2.00	19	0.97	17	0.0321
With aprepitant		(49.2%)		(54.5%)		(78.8%)
Ratio (D3 to D1)	19	1.57	19	0.760	13	1.36
p-value*		0.001		0.00003		0.02
Ratio (D3 to D2)	19	1.65	19	0.796	13	1.32
p-value*		0.0001		0.00001		0.02

Abbreviations: N, number of patients; Ratio (D3 to D1), the ratio of the geometric mean trough concentration of CR oxycodone plus aprepitant on day 3 to those of CR oxycodone alone on day 1; Ratio (D3 to D2), the ratio of the geometric mean trough concentration of CR oxycodone plus aprepitant on day 3 to those of CR oxycodone alone on day 2.
Geometric mean (% coefficient variance).
Values were corrected for dose, assuming that all patients received 20 mg of oxycodone.
*Paired t-test for difference between logarithmic geometric means (two-sided).
doi:10.1371/journal.pone.0104215.t004

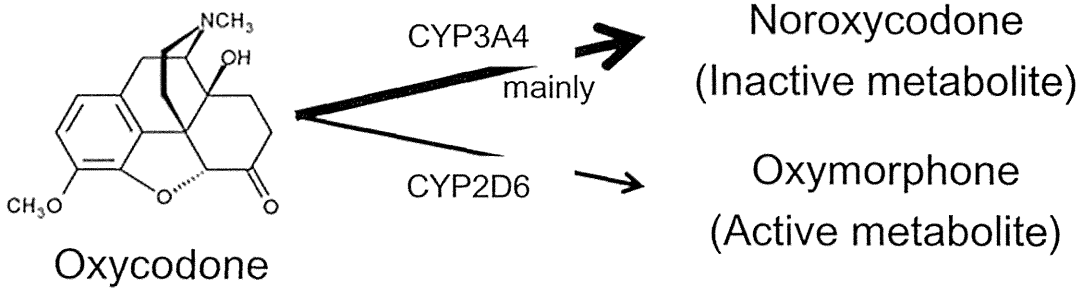

Figure 3. Metabolic pathway of Oxycodone.
doi:10.1371/journal.pone.0104215.g003

or 12 hours (Table 2). The median daily dosage of oxycodone was 20 mg (range, 10–60 mg) and the mean was 21.5 mg, with the median for each dosage being 10 mg (range, 5–20 mg) and the mean being 9.25 mg.

Oxycodone and its metabolites pharmacokinetics

All 20 patients were assessed for pharmacokinetics of oxycodone and its metabolites with and without aprepitant administration. In five patients who were administered with 5 mg of oral CR oxycodone every 12 hours, the plasma oxymorphone concentration was below the limit of quantification. Table 3 and 4 summarize the pharmacokinetic parameters of oxycodone administered alone or with aprepitant. Figure 2 shows the geometric mean plasma concentrations of oxycodone and its metabolites in patients (n = 6) who were administered with 10 mg of CR oxycodone every 12 hours alone or with aprepitant. The ratio of the geometric mean AUC_{0-8} and Cmax of CR oxycodone plus aprepitant [1,102 ng*hr/ml (CV 29.9%) and 2.79 ng/ml (CV 28.0%), respectively] to those of CR oxycodone alone [882 ng*hr/ml (CV 35.7%) and 2.28 ng/ml (CV 31.4%), respectively] was 1.25 (95% CI 1.14, 1.36; CV 21.8%; p = 0.00004) and 1.22 (95% CI 1.11, 1.34; CV 20.6%; p = 0.0002), respectively. The ratio of the geometric mean AUC_{0-8} of noroxycodone and oxymorphone with aprepitant [616 ng*hr/ml (CV 51.6%) and 20.7 ng*hr/ml (CV 65.8%), respectively] to those without aprepitant [718 ng*hr/ml (CV 45.2%) and 14.9 ng*hr/ml (CV 78.0%), respectively] was 0.86 (95% CI 0.81, 0.91; p = 0.00005) and 1.34 (95% CI 1.20, 1.49; p = 0.00004), respectively. The plasma concentrations of oxycodone and its metabolites were affected significantly by presence or absence of aprepitant.

The trough concentration of oxycodone and its metabolite on day 1 were similar to those on day 2, because steady state was reached. However, these trough concentrations with aprepitant on day 3 were higher than those on day 1 and day 2. The ratio of the geometric mean trough concentration of CR oxycodone plus aprepitant on day 3 to those of CR oxycodone alone on day 2 was 1.65 in oxycodone (p = 0.0001), 0.796 in noroxycodone (p = 0.00001), and 1.32 in oxymorphone (p = 0.02), respectively.

In this study and clinical practice, there was no increased incidence in pharmacologic effect and side effects of oxycodone due to concomitant use of aprepitant.

Discussion

The predominant metabolic pathway of oxycodone is CYP3A4-mediated N-demethylation to noroxycodone, while a minor proportion undergoes 3-O-demethylation to oxymorphone by CYP2D6 (Figure 3). This study demonstrated that inhibition of CYP3A4-mediated N-demethylation by aprepitant significantly increased the AUC of oxycodone by 25% and decreased the AUC of noroxycodone by 14%, while subsequently increasing the AUC of oxymorphone by 34% through alternating CYP2D6 pathway. We estimated in advance that a clinical meaningful significant level of interaction between oxycodone and aprepitant would be a 33% increase in the ratio of the geometric mean $AUC_{0\rightarrow8}$ under conditions where the CV was 45.1%. Essentially, the impact of aprepitant upon oxycodone was less than expected but the actual CV in the AUC of oxycodone was 30 and 35% in this study. Therefore, we consider that statistical significance was achieved as a result. In this study and clinical practice, there would be no increased incidence in pharmacologic effect and side effects of oxycodone due to concomitant use of aprepitant. We consider that a 25% increase (median 1.25; 95% CI 1.14, 1.36) in the ratio of the geometric mean $AUC_{0\rightarrow8}$ is a statistically significant effect, but that, due to its less extent than expected, at this time there is no need to change the CR oxycodone dose in clinical use of aprepitant in cancer patients, with adequate attention. With regard to oxymorphone which is an active metabolite, because oxymorphone is a potent opioid that has a 4 to 6 times lower μ-opioid receptor affinity and lower concentration than oxycodone [6] [14], an increase of oxymorphone would be unlikely to have a significant impact on the clinical relevance. However, because the recommended dose of aprepitant is 125-mg/80-mg regimen over 3 days, it is important to further investigate the possible effects of the 125-mg/80-mg aprepitant regimen on the pharmacokinetics of orally administered CR oxycodone in patients with cancer pain.

Aprepitant had no detectable inhibitory effect on the pharmacokinetics of intravenously administered docetaxel or vinorelbine [10] [11] but resulted in increased plasma concentration of orally administered dexamethasone or CR oxycodone [9]. It is expected that an orally-coadministered drug is affected to a greater extent by an inhibitory effect of intestinal CYP3A4 than intravenously-administered drug due to the higher intestinal concentration of aprepitant as compared to the plasma concentration. Therefore, we consider that this result for CR oxycodone may not be applicable to intravenously administered oxycodone. In this study, our patients received individual dose and schedule of CR oxycodone and combined with various anti-cancer agents according to standard treatment for their tumor types. Additionally, we didn't conducted placebo-controlled trial, because the primary endpoint in this study is not pharmacodynamics of oxycodone and its metabolites but pharmacokinetics. These are limitations of study, because this study was conducted in subjects whom continued to be administered CR oxycodone routinely for cancer pain. Further study to validate effects of aprepitant on the pharmacokinetics and pharmacodynamics of controlled-release oral oxycodone pharmacokinetic is expected.

The trough concentration of oxycodone and its metabolite on day 1 pre-dose were similar to those on day 2 pre-dose, despite these trough concentrations with aprepitant on day 3 were higher than those on day1 and day 2. This indicated that the trough concentrations of CR oxycodone alone at steady state were not observed inter-day variability (Table 4). Meanwhile, the ratio of the geometric mean AUC_{0-8} and trough concentration of CR oxycodone plus aprepitant to those of CR oxycodone alone was 1.25 (range 0.98–1.96) and 1.65 (range 0.54–3.41), respectively, with wide inter-patient variability observed (Figures S1 and S2). A pharmacogenomics study showed that a CYP2D6 genotype had an impact on plasma concentrations of oxycodone and oxymorphone, and the metabolism of oxycodone [15]. First, we are now planning a further pharmacogenomics study. Secondly, we will analyze plasma concentrations of aprepitant and investigate the possible influence of aprepitant concentrations on the pharmacokinetics of orally administered CR oxycodone.

In conclusion, aprepitant increased the exposure of oxycodone by 25% due to inhibiting its CYP3A4-mediated N-demethylation. The clinical use of aprepitant in patients receiving multiple doses of CR oxycodone for cancer pain significantly altered plasma concentration levels, but would not appear to need modification of the CR oxycodone dose in clinical co-administration of aprepitant in cancer patients, with adequate attention.

Supporting Information

Figure S1 Individual value plot of AUC_{0-8} of (A) oxycodone (n = 20), (B) noroxycodone (n = 20), and (C) oxymorphone (n = 15) in patients who were administered with CR oxycodone alone or with aprepitant. Dose of CR oxycodone: circle (5 mg), triangle (10 mg), square (15 mg), and pentagon (20 mg).
(TIF)

Figure S2 Individual value plot of trough concentration of (a) oxycodone (n = 19), (b) noroxycodone (n = 19), and (c) oxymorphone (n = 13) in patients who were administered with CR oxycodone alone or with aprepitant. Dose of CR oxycodone: circle (5 mg), triangle (10 mg), square (15 mg), and pentagon (20 mg).
(TIF)

Checklist S1 TREND Statement Checklist.
(PDF)

Protocol S1 Clinical Study Protocol (Japanese version).
(PDF)

Protocol S2 Clinical Study Protocol (English version).
(DOCX)

Acknowledgments

We greatly appreciate all patients who participated in this study and their families; the assistance of the staff of the Division of Medical Oncology/Hematology.

This work was supported by Yokoyama-Rinsyo foundation.

Author Contributions

Conceived and designed the experiments: YF, NC, HM. Performed the experiments: YF, MT, NC, NK, TS, YI, TM, HM. Analyzed the data: YF, MT, YI, HM. Contributed reagents/materials/analysis tools: YF, MT, HM. Wrote the paper: YF, MT, NC, YI, HM.

References

1. Kalso E (2005) Oxycodone. J Pain Symptom Manage 29: S47–56.
2. National Cancer Center. Cancer statistics in Japan 2010.
3. Leow KP, Smith MT, Williams B, Cramond T (1992) Single-dose and steady-state pharmacokinetics and pharmacodynamics of oxycodone in patients with cancer. Clin Pharmacol Ther 52: 487–495.
4. Poyhia R, Seppala T, Olkkola KT, Kalso E (1992) The pharmacokinetics and metabolism of oxycodone after intramuscular and oral administration to healthy subjects. Br J Clin Pharmacol 33: 617–621.
5. Leow KP, Cramond T, Smith MT (1995) Pharmacokinetics and pharmacodynamics of oxycodone when given intravenously and rectally to adult patients with cancer pain. Anesth Analg 80: 296–302.
6. Lalovic B, Phillips B, Risler LL, Howald W, Shen DD (2004) Quantitative contribution of CYP2D6 and CYP3A to oxycodone metabolism in human liver and intestinal microsomes. Drug Metab Dispos 32: 447–454.
7. Lalovic B, Kharasch E, Hoffer C, Risler L, Liu-Chen LY, et al. (2006) Pharmacokinetics and pharmacodynamics of oral oxycodone in healthy human subjects: role of circulating active metabolites. Clin Pharmacol Ther 79: 461–479.
8. Sanchez RI, Wang RW, Newton DJ, Bakhtiar R, Lu P, et al. (2004) Cytochrome P450 3A4 is the major enzyme involved in the metabolism of the substance P receptor antagonist aprepitant. Drug Metab Dispos 32: 1287–1292.
9. McCrea JB, Majumdar AK, Goldberg MR, Iwamoto M, Gargano C, et al. (2003) Effects of the neurokinin1 receptor antagonist aprepitant on the pharmacokinetics of dexamethasone and methylprednisolone. Clin Pharmacol Ther 74: 17–24.
10. Nygren P, Hande K, Petty KJ, Fedgchin M, van Dyck K, et al. (2005) Lack of effect of aprepitant on the pharmacokinetics of docetaxel in cancer patients. Cancer Chemother Pharmacol 55: 609–616.
11. Loos WJ, de Wit R, Freedman SJ, Van Dyck K, Gambale JJ, et al. (2007) Aprepitant when added to a standard antiemetic regimen consisting of ondansetron and dexamethasone does not affect vinorelbine pharmacokinetics in cancer patients. Cancer Chemother Pharmacol 59: 407–412.
12. Merck & Co I (2003) EMEND (aprepitant) capsules, for oral use. NJ, USA: Whitehouse Station.
13. Majumdar AK, McCrea JB, Panebianco DL, Hesney M, Dru J, et al. (2003) Effects of aprepitant on cytochrome P450 3A4 activity using midazolam as a probe. Clin Pharmacol Ther 74: 150–156.
14. Kalso E (2007) How different is oxycodone from morphine? Pain 132: 227–228.
15. Stamer UM, Zhang L, Book M, Lehmann LE, Stuber F, et al. (2013) CYP2D6 genotype dependent oxycodone metabolism in postoperative patients. PLoS One 8: e60239.

ANTICANCER RESEARCH *34*: 3337-3346 (2014)

TYRO3 as a Potential Therapeutic Target in Breast Cancer

ROUDY CHIMINCH EKYALONGO[1], TORU MUKOHARA[1,3,*], YOHEI FUNAKOSHI[1], HIDEO TOMIOKA[1], YU KATAOKA[1], YOHEI SHIMONO[1,2], NAOKO CHAYAHARA[1], MASANORI TOYODA[1], NAOMI KIYOTA[1] and HIRONOBU MINAMI[1,3]

[1]*Division of Medical Oncology/Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan;*
[2]*Division of Molecular and Cellular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe, Japan;*
[3]*Cancer Center, Kobe University Hospital, Kobe, Japan*

Abstract. *Aim: We evaluated the potential of TYRO3 as a therapeutic target in various types of breast cancer cell lines. Materials and Methods: The effects of TYRO3-knockdown by small interfering RNA (siRNA) on proliferation, cell-cycle distribution, and cell signaling in four estrogen receptor (ER)-positive/HER2-non-amplified (luminal-type), two ER-negative/HER2-amplified (HER2-type), and two ER-negative/HER2-non-amplified (triple negative [TN]-type) cell lines were compared. Results: Whereas TYRO3 knockdown induced the greatest proliferation suppression in luminal-type cells, and to a lesser extent in HER2-type cells, no proliferation inhibition was observed in TN-type cells. The TYRO3 siRNA-induced proliferation inhibition in luminal-type cells was observed in both estradiol (E2)-rich and -null conditions. The proliferation suppression was correlated with G0-G1/S cell-cycle arrest. Western blot analysis showed a decrease in phosphorylation of ERK1/2 or STAT3, and in cyclin D1 only in cell lines sensitive to TYRO3-knockdown. Conclusion: TYRO3 is a potential therapeutic target in breast cancer, particularly in luminal-type cells.*

With 1.6 million new cases annually, breast cancer is ranked as the primary cause of cancer-related deaths among women worldwide (5). The most challenging issue in breast cancer treatment remains metastasis, because the vast majority of these cases are incurable. Treatment outcomes resulting from endocrine therapy and/or chemotherapy are not satisfactory (8, 10); therefore, development of novel treatments is needed.

Correspondence to: Toru Mukohara, MD, D.Med.Sci., Department of Medical Oncology/Hematology, Kobe University Hospital, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Tel: +81 783825820, Fax: +81 783825821, e-mail: mukohara@med.kobe-u.ac.jp

Key Words: Breast cancer, TYRO3, siRNA, targeted therapy, luminal-type.

For the last decade, molecularly-targeted agents, particularly those against receptor tyrosine kinases (RTKs), have been applied in the treatment of advanced solid tumors. In breast cancer, lapatinib or trastuzumab, which target gene-amplified HER2 (13, 15), have contributed to the improved treatment options for HER2-positive breast cancer as single agents or in combination with cytotoxic agents. Despite the positive outcomes induced by HER2-targeted therapies, the therapeutic potential of many other RTKs is poorly understood.

In our previous study, we showed that a novel RTK, TYRO3, regulates the proliferation of MCF-7 breast cancer cells through controlling cyclin D1 expression (3). TYRO3 belongs to the TAM (TYRO3, AXL, and MER) receptor family, which shares the same ligand, growth arrest-specific 6 (GAS6). Binding of GAS6 to these subfamily members is known to control cell proliferation and survival through the PI3K/AKT/mTOR or RAF/MEK/ERK1/2 pathways (7, 11).

Among the TAM subfamily members, AXL is the one most closely studied regarding its relevance to malignant tumors. In fact, in breast cancer, AXL has been reported to be one of the most frequently overexpressed RTKs and to have prognostic potential (6). However, few studies have reported involvement of TYRO3 in tumorigenicity, and even less its potential as a new therapeutic target. Therefore, in the present study, we explored the therapeutic roles of TYRO3 in breast cancer using a panel of eight breast cancer cell lines.

Materials and Methods

Cell culture. Four estrogen receptor (ER)-positive/*HER2*-non-amplified (luminal-type; CAMA-1, T47D, MCF-7, and HCC-1428), two ER-negative/*HER2*-amplified (HER2-type; HCC-1419 and HCC-1954), and two ER-negative/HER2-non-amplified (triple-negative [TN]-type; MDA-MB-231 and HCC-1143) breast cancer cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were maintained

0250-7005/2014 $2.00+.40

in RPMI 1640 (Cellgro; Mediatech, Inc., Herdon, CA, USA) supplemented with 10% FBS (Gemini-Bio-Products, Inc., Woodland, CA, USA), 100 U/mL penicillin, 100 units/mL streptomycin, and 2 mM glutamine. Estradiol (E2) was not supplemented in the maintenance media. All cells were cultured at 37°C in a humidified atmosphere with 5 % CO_2 and were in logarithmic growth phase upon initiation of experiments.

TYRO3 small interfering RNA (siRNA) transfection. TYRO3 knockdown was performed using TYRO3 siRNA purchased from Invitrogen (Carlsbad, CA, USA). Optimal conditions for the gene knockdown were determined by multiple pilot experiments before transfection in accordance with the manufacturer's instructions, as previously reported (3). The sequences AACAAGUUUGGCCACG UGUGGAUGG (TYRO3 siRNA01) and AUCACUUCUUCCC ACUCCAAGAUGA (TYRO3 siRNA02) were selected for efficient knockdown. To minimize the risk of off-target effects in our siRNA analysis, the stealth RNAi negative-control low GC duplex (Invitrogen) was used in each knockdown experiment.

Cell proliferation assay. MTS assay kits were purchased from Promega (Madison, WI, USA). This kit determines the number of viable cells by a colorimetric method based on the bioreduction of 3-(4,5-dimethylthiazol-2yl)-5-(3-carboxyme-thowyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium (MTS) to a soluble formazan product detected spectrophotometrically at a wavelength of 490 nm. After 24 h of TYRO3 siRNA incubation, cells diluted in 10% FBS containing maintenance media without any antibiotics (160 μL/well) were plated in 96-well flat-bottom plates (day 0) (Corning, Inc., Corning, NY, USA) and a series of MTS analyses were performed from day 0 to 4 at 37°C in 5 % CO_2. The number of cells required to obtain an OD of 1.3-2.2, the linear range of the assay, was determined in a preparatory experiment (data not shown); 2,000 cells/well for CAMA-1, T47D, MCF-7, HCC-1428, HCC-1419, and HCC-1954, and 1,500 cells/well for MDA-MB-231 and HCC-1143. Each experiment was repeated at least three times. Analyses of the effect of 17β-E2 (Sigma-Aldrich, St. Louis, MO, USA) were conducted using phenol-free RPMI medium (Cellgro) without antibiotics, supplemented with 10 % charcoal-stripped FBS (Invitrogen). After 24 h of TYRO3 siRNA transfection, cells were seeded in 96-well flat-bottom plates, and 10 nM of E2 with and without 1 μM of fulvestrant (Sigma-Aldrich) were added and incubated for 72 h at 37°C in 5% CO_2 prior to MTS experiments.

Cell-cycle analysis. Cell-cycle analyses were performed as previously described (9). Briefly, 72 h after siRNA transfection, cells were trypsinized and cell-cycle distribution was assessed using a CycleTEST PLUS DNA Reagent Kit (Becton Dickinson [BD] Biosciences, Franklin Lakes, NJ, USA) in accordance with the manufacturer's instructions. Cells were analyzed by FACS Calibur flow cytometry (BD Biosciences) using CellQuest Pro (BD Biosciences) software and ModFit LTTM cell-cycle test software (Verity Software House Inc., Topsham, ME, USA). All experiments were repeated in triplicate.

Protein extraction and western blotting. Cells were washed once with ice-cold phosphate buffered saline and scraped immediately after adding lysis buffer (20 mM tris [pH 7.5], 150 mM sodium chloride, 2 mM EDTA, 10 % glycerol, 1 % NP40) containing protease and phosphatase inhibitors (100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 2 μg/mL aprotinin, 5 μg/mL leupeptin). Cell lysates were centrifuged at 14,000 × *g* for 10 min at 4°C to pellet-insoluble material, and the supernatant was collected as protein extract. Protein extract samples were separated using electrophoresis on 7.6 % SDS gels and transferred to polyvinylidene fluoride membranes, which were subsequently probed with primary antibodies. Phospho-AKT (Ser473), AKT, phospho-STAT3 (Tyr705) (D3A7), STAT3, TYRO3, and cyclin D1 (DCS6) were purchased from Cell Signaling Technology (Beverly, MA, USA). Phospho-ERK1/2 (pTpY185/187) and ERK1/2 (Invitrogen), anti-phosphoserine/threonine/tyrosine (Abcam, Cambridge, MA, USA), and β-actin (Sigma-Aldrich) were also purchased. Each targeted protein was detected using Amersham ECL plus Western Blotting Detection Reagents (GE Healthcare, Buckinghamshire, UK). The results are representative of at least two repeated experiments.

Immunoprecipitation. Protein A Dynabeads were purchased from Invitrogen. Following the manufacturer's instructions, 1,500 μg of protein extracts prepared as described previously were mixed with a TYRO3 antibody for 24 h at 4°C with rotation. The beads were washed three times in ice-cold washing buffer and mixed with the sample for 1 h at 4°C with rotation. After three washing cycles, the proteins were diluted in ice-cold elution buffer and LDS sample buffer and heated. The immunoprecipitation product was separated with SDS gels and subjected to western blotting, as described above.

Results

Effect of TYRO3 to proliferation of breast cancer cell lines. To determine the involvement of TYRO3 in the cell proliferation mechanism of breast cancer, we analyzed the effect of *TYRO3* gene silencing on eight breast cancer cell lines (four luminal-type, two HER2-type, and two TN-type). Treatment with TYRO3 siRNA resulted in the most prominent proliferation inhibition in the tested luminal-type cell lines (Figure 1A), particularly in MCF-7, T47D, and CAMA-1. When we treated the HER2-type cell lines, a significant but less extensive proliferation inhibition than in luminal-type cells was observed (Figure 1B). In contrast, *TYRO3* siRNA knockdown induced virtually no proliferation inhibition in TN-type cell lines (Figure 1C).

To further test if the TYRO3 siRNA-induced proliferation inhibition was TYRO3-specific, T47D cells were transfected with different concentrations of two TYRO3 siRNA duplexes. After 72 h, gradual inhibition of TYRO3 protein expression was obtained in a siRNA concentration-dependent manner (Figure 2). The degree of proliferation inhibition (Figure 2) was in parallel with that of TYRO3-knockdown. This finding supports that TYRO3 siRNA-induced proliferation inhibition was mediated by TYRO3 protein down-regulation.

Effect of TYRO3 knockdown on cell-cycle distribution. Since the results from the proliferation assays showed a clear impact of TYRO3 siRNA transfection by day 3 (Figure 1A and B), we decided to use 72 h as a time point to analyze the cell-cycle distribution of the siRNA-transfected cells. After exposure to TYRO3 siRNA for 72 h, an increase in the G_1-G_0