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IMAGING OF TUMOURS IN THE PAROTID REGION WITH INDIUM-111 LABELLED MONOCLONAL ANTIBODY REACTING WITH CARCINOEMBRYONIC ANTIGEN

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Abstract

Twenty-nine consecutive patients with palpable unilateral tumour in the parotid gland region were examined by planar scintigraphy at 22–28 h after injection of ^{111}In-labelled monoclonal anti-CEA-antibody. Two patients were also examined with emission tomography. Twenty-seven of the patients were operated on within 2–3 weeks. The serum CEA concentration was measured in 28 of the patients and was normal in all of them. Ten patients had positive scans and six of these had histologically malignant tumours; only four of the tumours with positive immunoscintigram were stainable immunohistochemically with anti-CEA. No patient with negative scintigram had parotid malignancy.

Key words: Parotid gland, tumours, carcinoembryonic antigen, monoclonal antibody, immunoscintigraphy.

The differential diagnosis of parotid gland tumours includes neoplasms, as well as inflammatory and developmental changes. In a study of fine-needle aspiration biopsy for diagnostics of head and neck malignancies, a false negative rate of 6.6% and false positive rate of 0% were reported (1). In another study 92% positive findings were found by aspiration cytology in 1 000 cases with histologically verified salivary gland tumours (2). When compared with histology, the cytology gave correct classification of the tumour in about 70% of the patients. Frozen sections of samples obtained during surgery are sometimes difficult to interpret and a false diagnosis of malignancy can lead to unnecessary radical surgery with sacrifice of the facial nerve. In one report, intraoperative frozen-section diagnosis agreed with the final diagnosis in only 63 out of 75 cases (3).

With radiological methods it is possible to obtain indirect information concerning the character of the tumour (4). Roentgensialography, ultrasonography, magnetic resonance imaging (MRI) and computer x-ray tomography (CT) can thus visualize the tumour. With these methods, however, it is difficult to draw conclusions about the probability of malignancy. Normal parotid lymph nodes (3–5 mm) were visualized in 7 of 30 patients by CT (5). In a recent low field MRI study on 103 patients with suspected salivary gland disease it was not possible to distinguish malignant lesions from chronic inflammatory disease (6). The ultimate diagnosis requires histology of the primary tumour.

Radionuclides have been used in the diagnosis of salivary gland tumours: ^{99}Tc^{m}-pertechnetate is accumulated in the normal salivary glands and also in Warthin's tumours (7). Accumulation of ^{111}In-labelled white blood cells in the lacrimal and salivary glands of a patient with Sjögren's syndrome has also been reported (8). There are other radioactive tracers, such as ^{67}Ga-citrate, which are accumulated by partly unknown mechanisms in malignant tumours including those in the parotid region. The efficacy of ^{67}Ga in the diagnosis of head and neck malignant neoplasms is limited by the normal uptake of this radionuclide in oral and pharyngeal minor salivary and secretory glands, which hampers the differentiation of malignant neck and head neoplasms from normal tissues (9). In pretherapeutic staging of head and neck malignancies, including salivary gland tumours, the only useful conventional radionuclide method is bone scintigraphy (10).

There are several applications of polyclonal or monoclonal radioantibodies in various malignancies (11, 12),

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but so far no reports on monoclonal radioantibodies for detecting malignant salivary gland tumours. This is probably due to the problem caused by the great variety of malignancies in the salivary glands, and to common serum tumour markers, such as CEA, rarely being elevated (13). The carcinoembryonic antigen (CEA) is produced by many different malignant cell lines, especially different adenocarcinomas and carcinomas of epithelial origin (14). An attempt to use radiolabelled polyclonal anti-CEA antibody for detection of head and neck malignancies has been reported in five patients (15).

The aim of the present study was to test a monoclonal antibody against CEA for imaging malignancies in the parotid area and compare the findings with the histology of the primary tumour. This was to our knowledge the first study where radioimmunoscintigraphy was used in non-selected consecutive patients with tumours of unknown type and normal serum tumour marker levels, and where the findings were confirmed histologically.

Material and Methods

A total of 29 consecutive patients (11 males, 18 females; 22–75 years, average 52 years) with a palpable tumour in the parotid gland region were examined. A control patient with no tumour in the parotid region was also examined. All patients gave their informed consent and the study was approved by the Ethical Committee of the Department of Otorhinolaryngology, Helsinki University Central Hospital. Three of the 29 tumours were later found not to be of parotid origin. Twenty-five of the patients underwent primary surgery of the palpable tumour. Furthermore, one local recurrence and one metastatic lesion were also operated. All operations were performed 2–3 weeks after immunoscintigraphy. Two patients were only followed up clinically, because the tumours decreased in size within 3 weeks. These tumours disappeared totally within 2 months, and were regarded as lymphadenitis.

Radioantibody. The $F(ab')_2$ fragment, a DTPA-conjugate of the anti-CEA-antibody (clone F023C5) (Indomab-K-2, Sorin Biomedica, Saluggia, Italy) was labelled with ^{111}In ($InCl_3$ from Amersham UK). (This IgG1 subclass antibody was prepared by the standard hybridoma technique using carcinoembryonic antigen purified from a hepatic matastasis of primary colon adenocarcinoma.)

The antibody dose was 0.3 mg and the injected activity of ^{111}In varied from 40 to 115 MBq. The labelling yield, measured in 8 preparations varied from 82 to 93%. Thin layer chromatography (ITLC SG, Gelman Sciences, Ann Arbor, Mich., USA) was only with 0.1 mol/l EDTA as solvent and the eluation time was approximately 5 min. The radioactivity was measured with a gamma counter.

Imaging procedure. All patients underwent scintigraphy 22–27 h after the injection of radioantibody. Planar spot images were made from the head (anterior and lateral views) with a General Electric Maxi 400 T gamma camera using a medium energy collimator. Three patients were also examined approximately 48 h after injection. In two patients also imaging with SPECT (single photon emission computed tomography) was performed 72 h after the injection. In the SPECT study 64 angles were recorded with one frame per 35 s. The camera, connected to a PDP-11 computer fitted with GAMMA-11/34 software, had a resolution of 350 dots and a colour display. The parotid regions were analyzed using a region-of-interest technique (ROI); the pulses were calculated in a square in both parotid regions and the tumour-to-background ratio was calculated after correction for background.

Surgery. Twenty-seven patients were operated on and a superficial parotidectomy was performed on all, except for three patients who underwent total parotidectomy. Patients' descriptions, size of tumour and histopathological diagnoses are listed in the Table.

Immunohistochemistry. The scintigraphically positive tumours (10/27) were stained for CEA (Table). The immunohistochemically positive tumours were acinic cell carcinomas (2 patients), metastasis of an adenocarcinoma (possibly of renal origin) and adenoid cystic carcinoma.

Serum CEA. Serum samples in 28 patients were taken just before injecting the radioantibody. The concentration of CEA in serum was measured with an RIA method.

Human anti-murine-antibodies. Serum samples for the assay of human anti-murine-antibodies (HAMAs) were taken in 18 patients within 2 weeks to 4 months after the radioantibody injection. Additional serum samples after 2–3 months were taken in three patients; in 14 patients the sera were taken within 2–4 weeks. The IgG and IgM subclass HAMAs were determined with an ELISA method (Enzygnost-HAMA-Micro, Behringwerke, Marburg, West Germany).

Results

No adverse reactions to the radioantibody were observed. The results are presented in the Table. Ten of the imaged 60 parotid gland regions were positive. Six of these positive findings corresponded to a malignant tumour: two acinocellular carcinomas, one adenoid cystic carcinoma, one metastasis of adenocarcinoma of unknown origin, one soft tissue sarcoma and one paraganglioma. The paranganglioma (a carotid body tumour) was considered malignant because of its potential fatal course and ability to metastasize. There were four non-malignant tumours with positive scan. There were no negative scintigrams associated with malignancy. The control patient without parotid tumour had no pathologic uptake in the head region. Fig. 1 presents planar lateral views from a patient with an acinocellular carcinoma. SPECT study of hemangiopericytoma is presented in Fig. 2 and planar images of adenoid cystic carcinoma in Fig. 3.

Table

Histological diagnosis, tumour diameter, serum CEA concentration and ^{111}In-anti-CEA radioimmunoscintigraphy results in patients with parotid gland region disorders

Pat. No. age/sex	Histology	Diameter (cm)	S-CEA μg/l	Scintigraphy TBR/vis.
1 43/F	Lipoma	2	<3	1.0 neg.
2 70/F	Adenolymphoma	2, 2, 2*	<3	1.4 pos.
3 44/M	Non-neoplastic cyst	2	<3	1.0 neg.
4 53/M	Tuberculosis	3	<3	1.0 neg.
5 22/M	Lymphadenitis?	1**	<3	1.1 neg.
6 53/F	Lymphadenitis?	1.5**	<3	1.1 pos.
7 42/M	Lymphadenitis	2	<3	0.9 neg.
8 57/M	Mikulicz' disease	1.5	<3	1.0 neg.
9 74/M	Mikulicz' disease	2	<3	1.0 neg.
10 57/F	Mikulicz' disease	2.5, 1.5*	<3	1.4 pos.
11 75/M	Pleomorphic adenoma	3.5	<3	1.0 neg.
12 59/F	Pleomorphic adenoma	1.5	<3	1.0 neg.
13 72/F	Pleomorphic adenoma	2	<3	1.5 pos.
14 59/F	Pleomorphic adenoma	2	<3	1.1 neg.
15 40/F	Pleomorphic adenoma	1	<3	1.0 neg.
16 45/F	Pleomorphic adenoma	3	<3	1.1 neg.
17 62/F	Pleomorphic adenoma	3	<3	1.0 neg.
18 69/F	Pleomorphic adenoma	1.5	<3	1.0 neg.
19 67/F	Pleomorphic adenoma	1	<3	1.0 neg.
20 32/F	Pleomorphic adenoma	1.5	<3	1.1 neg.
21 56/F	Pleomorphic adenoma	3.5	3.7	1.0 neg.
22 33/F	Pleomorphic adenoma	1.5	<3	0.9 neg.
23 32/F	Pleomorphic adenoma	1.5	<3	1.0 neg.
24 66/M	Paraganglioma	3	<3	1.6 pos.
25 50/F	Acinic cell ca	3.5	nd	1.2 pos.
26 37/M	Acinic cell ca	1	<3	1.3 pos.
27 56/F	Adenoid cystic ca	2	<3	1.2 pos.
28 27/M	Hemangiopericytoma	4	<3	2.2 pos.
29 67/M	Met of adenoca	2	nd	1.2 pos.
30 34/M	Control	no tumour	<3	1.0 neg.

Abbreviations:
? = not histologically verified (clinical diagnosis)
* = multiple solitary tumours (diameters indicated)
** = palpation findings (approx. diameters indicated)
ca = carcinoma met = metastasis nd = not done
TBR = tumour-to-background ratio vis = visual interpretation

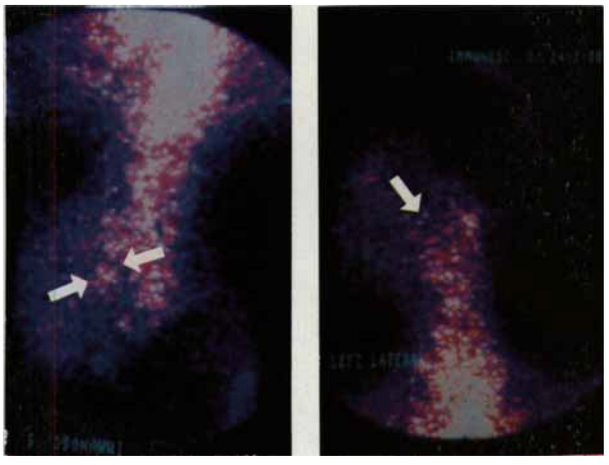

Fig. 1. Lateral immunoscintigrams of a patient with an acinic cell carcinoma in the right parotid gland. a) Right side. b) Left side.

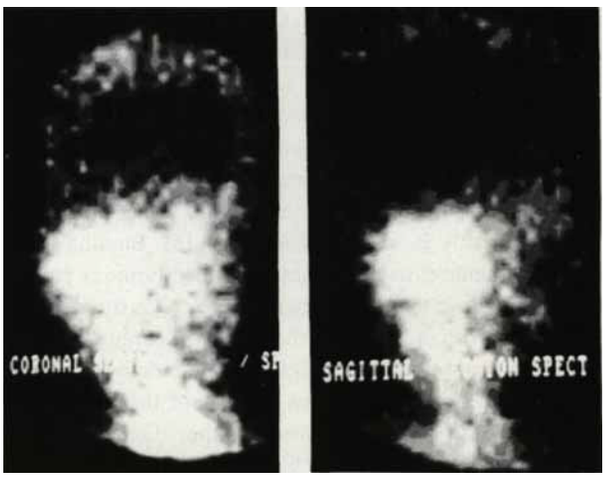

Fig. 2. A large uptake in the right parotid region in a patient with hemangiopericytoma (visualized by SPECT) a) Coronal section. b) Sagittal section.

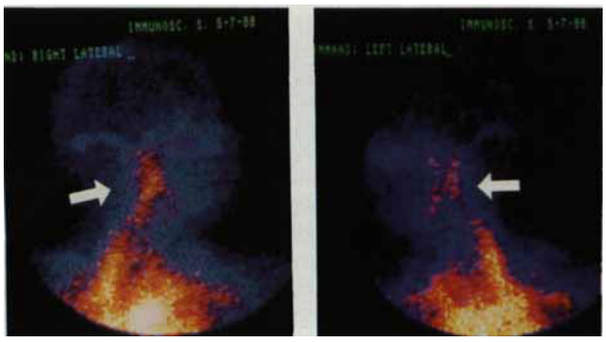

Fig. 3 Lateral immunoscintigrams of a patient with an adenoid cystic carcinoma in the right parotid gland. a) Right side. b) Left side.

The serum CEA concentrations are presented in the Table and were below 4.0 μg/l. The tumour-to-background ratios (TBR) were relatively low even when the scintigrams, by visual interpretation, were regarded as positive; however, in one case TBR was >2.0. The findings were regarded as positive if this ratio exceeded 1.2. The TBRs are presented in the Table with the corresponding results from visual interpretation of the immunoscintigram. The findings were considered suspicious when TBR was 1.1–1.2. These lesions were analyzed carefully with the ROI technique and were considered positive only if the subjective evaluation, performed independently by the two authors, was positive.

Trends of elevated HAMA of IgG subclass and IgM subclass were observed in 3 and 16 of 20 cases respectively.

Discussion

In this study we have shown that it is possible to distinguish malignant and benign parotid tumours by anti-CEA immunoscintigraphy. Our material, however, is small, and only five different types of malignancy were represented.

The mechanism of antibody uptake is not apparent, because there was no clear correlation between immunohistochemical and immunoscintigraphic findings. It must be recognized that anti-CEA antibodies sometimes behave very differently in vitro and in vivo (16). Binding of the $F(ab')_2$ fragment to the tumour cell membrane is perhaps unspecific close to the Fc-fragment cleavage site. Another type of unspecific binding has actually been shown to occur as unspecific human IgG is accumulated in inflammatory and neoplastic lesions because of the Fc-fragment (17). One supposed mechanism for the ^{67}Ga-citrate accumulation in malignant neoplasms is increased vascular permeability (18), but this issue is still controversial (19). This mode of uptake could also be valid for antibodies. Furthermore, the latter is normally accumulated in the salivary glands, whereas ^{111}In-labelled anti-CEA antibodies do not show any affinity for normal salivary glands.

Two cases with multiple lesions were considered false positive. One reason for the positive scintigrams might be superpositioning of tumours containing non-binding circulating antibody. One of the four false positive findings was probably caused by infection, because the parotid mass disappeared during the follow-up; anti-CEA antibodies can recognize structures on the granulocyte cell surface or granulocytes can entrap antibody by pinocytosis. An anti-granulocyte antibody was originally raised against carcinoembryonic antigen and it was occasionally observed to bind to granulocytes (20).

The tuberculoma in our material was negative although it has been possible to image such a tumour in mice with another monoclonal antibody (21). The pleomorphic adenomas had negative immunoscintigraphy with one exception (Fig. 4). These tumours can be diagnosed with MRI and the differential diagnosis of Mikulicz's disease, parotitis and cysts is also possible with MRI (7). The importance of paramagnetic contrast media in MRI imaging of parotid tumours is still unknown; Gd-DPTA facilitated the interpretation of results in paragangliomas (22).

The immunoscintigraphy method described by us is atraumatic and easy to perform, and the immunogenicity

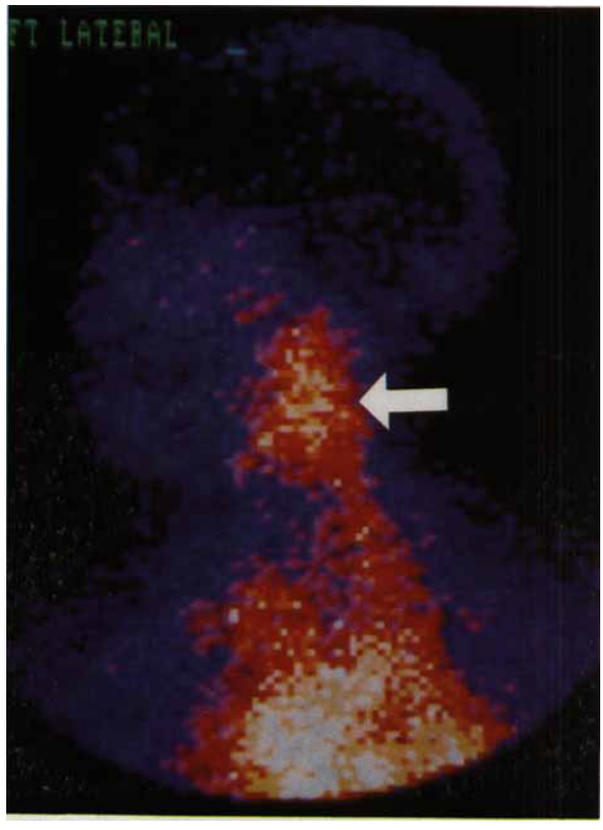

Fig. 4. A positive immunoscintigram (left lateral view) of a patient with pleomorphic adenoma on the left side.

of mouse proteins can be avoided, at least partly, by producing human monoclonal antibodies or chimeric antibodies. The serum CEA concentration did not correlate with our immunoscintigraphy findings. A similar discrepancy was reported by Mach et al. (23). This indicates that serum CEA is not a useful marker in parotid malignancies, even though radiolabelled anti-CEA is a promising diagnostic tool. However, other monoclonal antibodies also have to be tested in larger series of various malignancies. CEA is expressed by many types of tumours (colorectal, breast, lung, pancreatic, and cervical carcinomas) and specificity causes problems that must be recognized when imaging parotid malignancies of various origin.

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