previously treated adanced epithelial ovarian cancer: activity in platinum-resistant disease. *J Clin Oncol* 1992, **10**, 243–248.
7. Aapro MS, Pujade-Lauraine E, Lhomme C, *et al.* EORTC Clinical Screening Group: Phase II study of Taxoter[R] (docetaxel) in ovarian cancer. *Proc Am Soc Clin Oncol* 1993, **12**, 256.
8. Einzig AI, Wiernik PH, Sasloff J, *et al.* Phase III study and long term follow-up of patients treated with Taxol for advanced ovarian adenocarcinoma. *J Clin Oncol* 1992, **10**, 1748–1753.
9. WcGuire WP, Rowinsky EK, Rosenshein NB, *et al.* Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. *Ann Intern Med* 1989, **111**, 273–279.
10. Thigpen T, Blessing J, Ball H, *et al.* Phase II trial of Taxol as second line therapy for ovarian carcinoma: a Gynecologic Oncology Group Study, *Proc Am Soc Clin Oncol* 1990, **9**, 156.
11. Trimble EL, Adams JD, Vena D, *et al.* Paclitaxel for platinum-refractory ovarian cancer: results from the first 1,000 patients registered to National Cancer Institute Treatment Referral Center 9103. *J Clin Oncol* 1993, **11**, 2405–2410.
12. Ten Bokkel Huinink WW, Eisenhauer E, Swenerton K, for the Canadian–European Taxol Cooperative Trial Group. Preliminary evaluation of a multicenter randomized comparative study of Taxol[R] (paclitaxel) dose and infusion length in platinum-treated ovarian cancer. *Cancer Treat Rev* 1993, **19** (suppl.C), 79–86.
13. Guastalla JP, Lhomme C, Dauplat J, *et al.* Taxol[R] (paclitaxel) safety in 99 patients pretreated by platinum chemotherapy for ovarian carcinoma. *Proc Am Soc Clin Oncol* 1994, **13**, 270.
14. Vermorken JB, Huijskes RVHP, van Rijswik REN, *et al.* "Paclitaxel or no paclitaxel in second line, that's the question" in ovarian cancer patients who relapse after a prolonged platinum-free interval. 8th NCI-EORTC symposium on new drugs in cancer therapy. Amsterdam, 1994 abstract no. 497 p. 199.
15. Kukelda A, Tresukosol D, Edwards C, *et al.* Post taxoid carboplatin reinduction in patients with platin refractory epithelial ovarian cancer. *Proc Am Soc Clin Oncol* 1994, **13**, 276.

Pergamon

European Journal of Cancer Vol. 30A, No. 12, pp. 1741–1744, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0959–8049/94 $7.00+0.00

0959-8049(94)00238-X

Papers

Local Injection of OK-432/Fibrinogen Gel into Head and Neck Carcinomas

H. Kumazawa, T. Yamashita, T. Tachikawa, M. Minamino and Y. Nakata

Immunotherapy with biological response modifiers (BRM) is a possible strategy against head and neck solid tumours. However, the rapid disappearance of BRM from the tumour area is one of the reasons for its limited clinical application. In this pilot study, fibrinogen gel containing OK-432 (a compound composed of attenuated *Streptococcus pyogenes*), an inducer of natural killer cells and T-cell cytotoxity, was injected directly into head and neck solid tumours of 15 patients. A dose of 5 Klinische Einheiten (KE) of OK-432 was reconstituted in 1 ml aprotinin and mixed with fibrinogen, the latter to maintain the OK-432 locally. 3 patients showed tumour regression, and in addition, we observed histological changes in the injected tumour of all patients. These results suggest that OK-432/fibrinogen gel generates a local immune response, leading to tumour regression.

Keywords: immunotherapy, head and neck carcinoma
Eur J Cancer, Vol. 30A, No. 12, pp. 1741–1744, 1994

INTRODUCTION

OK-432, A LYOPHILISED powder prepared from a penicillin G-treated Su strain of Type III Group A *Streptococcus pyogenes*, has been shown to manifest a tumoricidal effect not only directly, but also by potentiating the host's immunity [1, 2]. Talmadge and Herberman reported that OK-432 is a potent biological response modifier (BRM), which can augment natural killer (NK) cell activity, macrophage-mediated cytotoxity and T-cell function [3]. In Japan, the subcutaneous systemic injection of OK-432 is considered to be an effective clinical treatment in a substantial proportion of patients with various tumours, resulting in improved survival [4, 5].

Recent interest in OK-432 has focused on its effect when administered locally. However, the duration of response is limited and local intratumoral administration of OK-432 does not always provide the desired effect on solid tumours, unless it is repeatedly administrated in large doses [6, 7]. One reason for

Correspondence to H. Kumazawa.
H. Kumazawa, T. Yamashita, T. Tachikawa and M. Minamino are at the Department of Otolaryngology, Kansai Medical University, Fumizonochol, Moriguchi City, Osaka 570; and Y. Nakata is at the Department of First Pathology, Hyogo Medical University, Japan.
Revised 29 Apr. 1994; accepted 6 June 1994.

its clinical limitation may be the rapid release and disappearance of the BRM from the tumour area injected.

To overcome the shortcomings of intratumoral injection of OK-432, we injected OK-432 mixed with fibrinogen solution into the tumour mass, as proposed by Monden [8]. The intratumoral injection of OK-432 in combination with the fibrinogen gel results in rapid formation of a fibrin mesh which can trap OK-432 and prolong the duration of its anti-tumour effect, since the procoagulants secreted from tumour tissue can convert fibrinogen to fibrin.

In this clinical trial, in which OK-432/fibrinogen gel was injected into the tumour mass of 15 patients with advanced head and neck carcinoma, we assessed the clinical usefulness of local treatment.

PATIENTS AND METHODS

The characteristics of 15 patients with advanced head and neck carcinomas are listed in Table 1. The site of injection of OK-432/fibrinogen gel was the primary tumour in 4 patients and recurrent tumour in 11 patients, 2 with local recurrence and 9 with metastasis. The histological diagnosis of all carcinomas was squamous cell carcinoma, except for case 14 which was an adenocarcinoma. All patients were informed about the treatment and side-effects prior to enrolment in this clinical pilot study.

The OK-432/fibrinogen gel was prepared according to the method of Monden [8]. Briefly, 5 Klinische Einheiten (KE) of OK-432 (Chugai Pharmaceutical Co. Ltd, Tokyo, Japan), corresponding to 0.5 mg of lyophylised *Streptococcus pyogenes*, were dissolved in 1 ml of aprotinin (1000 kallikrein inhibitor units), and mixed with 80 mg of heat-treated human fibrinogen. The fibrinogen used was a component of a commercially-available fibrin glue (Beriplast P; Boehringer Werke AG, Marburg, Germany). The fibrinogen contained a relatively high concentration of factor XIII, sufficient to form a fibrin mesh, corresponding to 60 times the factor XIII activity in 1 ml of normal plasma. Both fibrinogen and factor XIII were pasteurised at 60°C for 10 h, and tested to be free of viral contamination. Aprotinin, another component of the fibrin glue, was used to protect the fibrin clot from fibrinolytic enzymes, because tumour cells have been reported to possess a high level of fibrinolytic activity.

One millilitre of OK-432/fibrinogen gel was injected into the tumour mass using a small syringe. To avoid the pain during the injection, local anaesthesia with 0.5% lidocaine was performed before the injection of OK-432/fibrinogen gel. The injection of OK-432/fibrinogen gel was performed one to three times. The specimens obtained were stained with haematoxylin and eosin after fixation in 10% formalin. Each resected tumour was examined at the time after injection. Macroscopic changes of 5 cases (cases 7, 8, 9, 12 and 14) could not be evaluated due to previous multiple samplings for histopathological examination, resulting in insufficient volumes being left to measure a tumour response.

RESULTS

In 3 patients (cases 1, 2 and 15), marked tumour regression was observed within 7 days. Figure 1 shows the macroscopic regression of the recurrent tumour of case 2 after the injection of OK-432/fibrinogen gel. Tumour in the left nostril regressed after the injection (Figures 1A, B). Histological observation showed degenerative tumour tissue and infiltration of neutrophils, macrophages and lymphocytes after the injection (Figures 1C, D). Macroscopic changes of 5 cases (case 7, 8, 9, 12 and 14) were not evaluated due to previous multiple sampling for histopathological examination, which demonstrated the infiltration of macrophages within 2–3 days of injection, but a large number of lymphocytes and neutrophils between 7 and 14 days. The other patients did not show any macroscopic changes. However, these tumours did not show progressive growth. In all cases, we observed degeneration of tumour tissue and infiltration of neutrophils, macrophages and lymphocytes (Table 1). An interesting finding was that polynuclear giant cells were frequently observed within 7 days after injection. We also observed necrotic changes of tumour tissues and the formation of granulation containing few tumour cells around 7–15 days after injection.

Table 1. Clinical characteristics of patients

Patients no.	Age (years)	Sex	Site of primary tumour	Stage or recurrence (metastatic site)	Treatment of OK-432	Histological change after injection: Lymphocyte infiltration	Histological change after injection: Tumour degeneration
1	76	M	Trachea	Recurrence (neck)	5 KE × 3	+	++
2	77	M	Maxillary sinus	Recurrence (nose)	5 KE × 3	++	++
3	64	M	Tonsil	T2N0M0	5 KE × 1	+	++
4	30	M	Tongue	T3N0M0	5 KE × 2	+++	+
5	70	F	Hypopharynx	T3N0M0	5 KE × 1	+	+
6	54	F	Larynx	Recurrence (neck)	5 KE × 1	++	+
7	61	F	Hypopharynx	Recurrence (neck)	5 KE × 1	+	+++
8	61	M	Floor of mouth	Recurrence (neck)	5 KE × 1	++	++
9	61	M	Tongue	Recurrence (neck)	5 KE × 1	++	++
10	45	M	Tongue	T3N0M0	5 KE × 1	++	+
11	64	M	Hypopharynx	Recurrence (neck)	5 KE × 1	+	+
12	55	M	Maxillary sinus	Recurrence (neck)	5 KE × 1	++	++
13	75	M	Hypopharynx	Recurrence (neck)	5 KE × 1	++	++
14	86	M	Maxillary sinus	Recurrence (nose)	5 KE × 3	+	+
15	75	M	Tongue	Recurrence (neck)	5 KE × 1	++	+

Lymphocyte infiltration was evaluated according to the criteria of Shimokawara [9]. −, absent; +, slight; ++, moderate; +++, marked. Tumour degeneration was evaluated as: −, no degeneration; +, slight; ++, moderate; +++, marked change. KE, Klinische Einheiten.

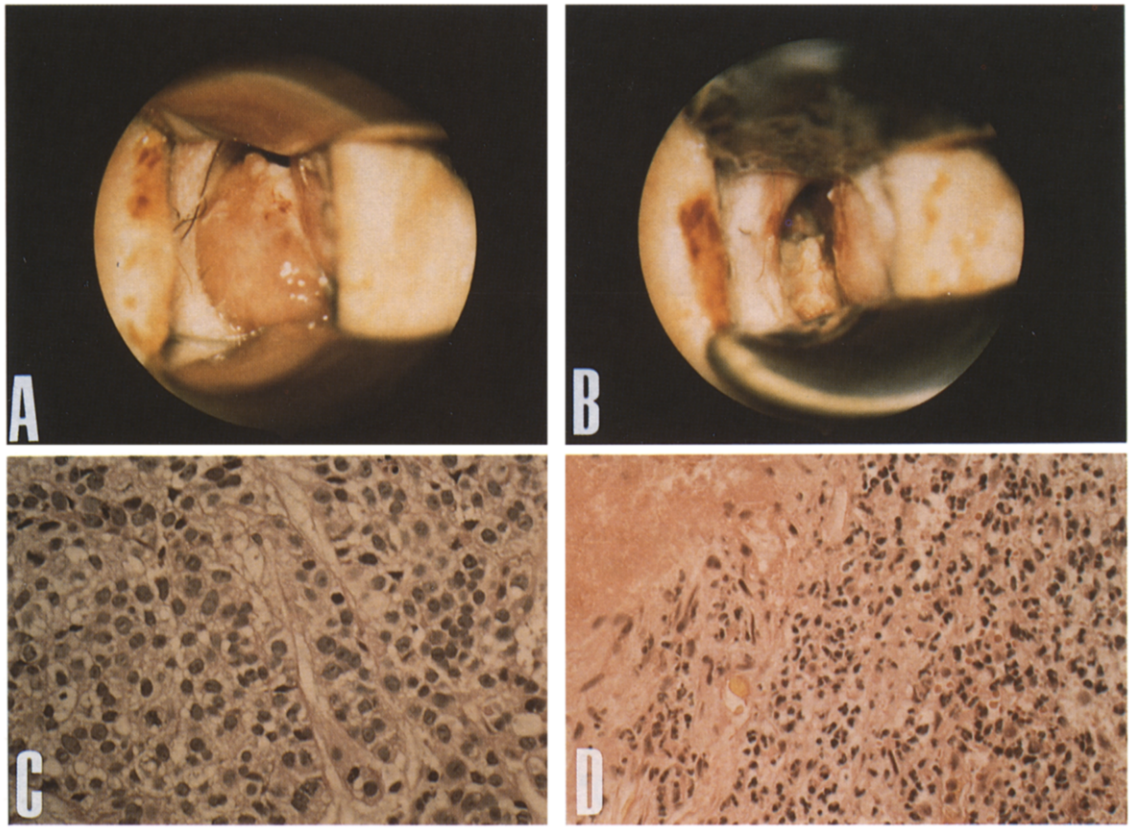

Figure 1. Nasal tumour in a 77-year-old man before local injection of OK-432/fibrinogen gel (A). Tumour regression of nasal tumour after the injection (B). Histological change of tumour before local injection of OK-432/fibrinogen gel (C) and after local injection (D) (haematoxylin-eosin, magnification × 400).

The usual side-effects of OK-432 were observed on the day of injection, with 6 patients having fever above 40°C and chills. However, this fever was resolved within 24 h. One patient (case 10) showed unusual elevations of glutamic-oxalocenic transferase and glutamic pyruvic transaminase, which normalised within 7 days.

DISCUSSION

Our clinical experience showed that local immunotherapy with OK-432/fibrinogen gel resulted in tumour regression in 3 of the 15 patients. Histological examination demonstrated tumour degeneration and lymphocyte infiltration at the site of injection in all cases.

These findings suggest that an important contribution to tumour degeneration may be the physiological gelatinising of OK-432 with fibrinogen to prolong the duration of the BRM at the site of injection. Exogenous injected fibrinogen was considered useful locally retaining the OK-432, because endogenous fibrinogen is thought to be consumed in tumour stroma. The procoagulants secreted from tumour tissue can convert exogenous fibrinogen to fibrin. The concentration of fibrinogen used in this study was more than 20 times that in plasma, which was sufficient to stabilise fibrin by crosslinking.

The first stage for tumour degeneration after injection of OK-432/fibrinogen gel is suggested to be macrophage accumulation. The marked accumulation of macrophages in the tumour stroma was thought to be a part of an inflammatory response. Macrophages phagocytosed OK-432 in the early phase, became activated and formed polynuclear giant cells up to 7 days after the injection. These activated macrophages could trigger the onset of delayed-type hypersensitivity in the intermediate to late phases of inflammation, causing the accumulation of neutrophils. Subsequently, necrotic change of tumour tissue and the formation of granulation, containing a few tumour cells, were observed.

These phenomena are similar to Monden's observations [8] that OK-432 provokes severe inflammation in the tumour stroma and induces immune responses that can contribute to granulomatous hypersensitivity as the final stage of inflammation. According to Monden's report, the host would recognise the tumour as wound tissue after development of marked inflammation due to OK-432 injected in the tumour mass. The wound healing induced by granulomatous hypersensitivity results in degeneration of tumour stroma.

Another consideration in tumour regression is the augmentation of natural killer (NK) cell activity, cytotoxic macrophages and autologous tumour cell killing at the site of the tumour. These possibilities have been suggested by both *in vivo* and *in vitro* assays [2, 3, 10]. Uchida demonstrated augumentation of NK cell activity, lymphokine-activated killer (LAK) cell activity and tumour-associated cytotoxic T-lymphocytes following systemic and local administration of OK-432 [4, 11]. Since OK-432 is a potent inducer of cytokines, such as interleukin-1 (IL-1), IL-2 and interferon (IFN)-γ [12], OK-432 may be a prerequisite to the appearance of cytotoxic T-lymphocytes and LAK cells at the tumour site, observed after OK-432 treatment.

From a clinical point of view, an important question remains regarding the impact of immunotherapy with OK-432/fibrinogen gel on survival, both in responders and overall. Cases 1, 2 and 15, who showed tumour regression after injection of OK-

432/fibrinogen gel, did not survive for more than 3 years, although they were subsequently treated with chemotherapy or radiation therapy. The other 4 cases of primary carcinomas (cases 3, 4, 5 and 10) and 8 of the recurrent carcinomas (cases 6–9, 11–14), which were unaffected by OK-432, were also treated with surgery and/or palliative chemotherapy plus radiation therapy after local injection of OK-432/fibrinogen gel. It is difficult to assess the clinical benefit of applications of OK-432/fibrinogen gel because of the small number of patients and the short observation time in this trial. However, it is relatively easy to apply multiple, local treatments to tumours existing in the head and neck in comparison with tumours of other organs. In this respect, a multi-institutional collaborative study would clarify the clinical applicability of local administration of OK-432/fibrinogen gel to head and neck carcinomas.

1. Okamoto H, Shoin S, Koshimura S, *et al.* Studies on the anticancer and streptolysin-forming abilities of hemolytic streptococci. *Jpn J Microbiol* 1967, **11**, 326–336.
2. Nio Y, Zighelboim J, Bonavida B, *et al.* Sensitivity of fresh and cultured ovarian tumor cells to tumor necrosis factor, interferon-alfa 2 and OK-432. *Cancer Immunol Immunother* 1988, **27**, 246–254.
3. Talmadge J, Herberman RB. The preclinical screening laboratory; evaluation of immunomoderatpry and therapeutical properties of biological response modifiers. *Cancer Treat Rep* 1986, **70**, 171–182.
4. Uchida A, Hoshino T. Clinical studies on cell-mediated immunity in patients with malignant disease: I. Effect of immunotherapy with OK-432 on lymphocyte subpopulation and phytomitogen responsiveness *in vitro*. *Cancer* 1980, **53**, 478–483.
5. Yamamura Y, Yasue M, Nakazono H, *et al.* A controlled study of surgical adjuvant chemoimmunotherapy with FT-207 and OK-432 for advanced stomach cancer: fourth study. Clinical application of OK-432 for control of cancer. *Excerpta Med* 1986, **80**, 5397–5401.
6. Toge T, Kuninobu H, Yamaguchi Y, *et al.* The clinical efficacy of intratumoral OK-432 administration in advanced cancer patients. *Jpn J Surg* 1988, **18**, 668–674.
7. Tsujitani S, Okamura T, Bab H, *et al.* Endoscopic intratumoral injection of OK-432 and Langerhans' cells in patients with gastric carcinoma. *Cancer* 1988, **61**, 1749–1753.
8. Monden T, Morimoto H, Shimano T, *et al.* Use of fibrinogen to enhance the anti-tumor effect of OK-432. *Cancer* 1992, **69**, 636–642.
9. Shimokawara I, Imamura M, Yamanaka N, *et al.* Identification of lymphocyte subpopulations in human breast cancer tissue and its significance. *Cancer* 1982, **49**, 1456–1464.
10. Saito M, Nanjo M, Aonuma E, *et al.* Activated macrophages are responsible for the tumor inhibitory effect in mice receiving intravenous injection of OK-432. *Int J Cancer* 1984, **33**, 271–276.
11. Uchida A, Micksche M. Lysis of fresh tumor cells by autologous peripheral blood and pleural effusion lymphocytes activated by OK-432. *J Natl Cancer Inst* 1983, **71**, 673–680.
12. Christmas SE, Meager A, Moore M. Studies of the enhancement of natural cytotoxity by the streptococcal immunopotentiator OK-432. *Int J Immunopharmacol* 1986, **8**, 83–89.

Pergamon

European Journal of Cancer Vol. 30A, No. 12, pp. 1744–1751, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0959–8049/94 $7.00+0.00

0959-8049(94)00330-0

One-year follow-up of the 'Starting Again' Group Rehabilitation Programme for Cancer Patients

G. Berglund, C. Bolund, U.-L. Gustafsson and P.-O. Sjödén

In a randomised prospective study, a brief structured rehabilitation programme, 'Starting Again', was evaluated over a follow-up year. 98 patients were assigned to the programme, and 101 to the control condition. The 11, 2-h sessions emphasised physical training, information and coping skills. Patients in the programme improved significantly more than the controls with respect to appraisal of having received sufficient information, physical training, physical strength and fighting spirit. Results indicate improvement with respect to the three areas focused on in the 'Starting Again' programme: physical training, information and coping skills training.

Key words: rehabilitation, group, randomisation, coping, patient information, physical training, long-term follow-up

Eur J Cancer, Vol. 30A, No. 12, pp. 1744–1751, 1994

INTRODUCTION

SEVERAL REVIEWS of psychosocial intervention studies with cancer patients have been published during the last decade [1–4]. Watson [3] carefully and methodically reviewed work published before 1983. Telch and Telch [2] critically examined efforts to promote coping with cancer, and Mathieson and Stam [1] scrutinised the relevance of psychosocial interventions for cancer patients. In the most recent review, Andersen [4] made a distinction between psychological interventions for low, moderate and high risk morbidity patients. This distinction is potentially important for the evaluation of interventions, since the rate of spontaneous recovery may vary