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Randomised Study of Immunotherapy with OK-432 in Uterine Cervical Carcinoma

Fumitaka Kikkawa, Michiyasu Kawai, Hidenori Oguchi, Masayoshi Kojima, Hisatake Ishikawa, Mitsumasa Iwata, Osamu Maeda, Yutaka Tomoda, Yoshitaro Arii, Kazuo Kuzuya, Masahiro Ohta, Takao Ishizuka, Sen-Ei Hattori and Kunio Aoki

OK-432, a streptococcal preparation, was administered to patients with stage Ib and II cervical carcinoma except for adeno- and adenosquamous carcinomas. To evaluate the efficacy of OK-432 precisely, 177 patients were stratified by clinical stage, radiotherapy, and lymph node metastasis after complete radical hysterectomy and pelvic lymphadenectomy. Within each stratum, patients were divided randomly into OK-432 and control groups. 85 patients received OK-432 and 92 patients did not. No significant difference was observed in overall 5-year disease free rates between the OK-432 and the control groups, although the mean diameter of erythema on SU-polysaccharide (SU-PS) skin test was larger in the OK-432 group than in the control group. In stage IIb, a significant difference was observed between the OK-432 and control groups. This difference, however, could be attributed in part to the different incidence of the lymph node metastasis. In stage II without lymph node metastasis, 5-year disease free rate was significantly higher in the OK-432 group.
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INTRODUCTION

CARCINOMA OF the uterine cervix is the most common cancer among gynaecological malignant tumours in Japan. The decrease in mortality from cervical cancer during the past 25 years has been attributed to the detection of early stage cancer rather than to improvement in the treatment of invasive cancer, since screening for uterine carcinoma has become widespread. Five-year survival rate is 99% for carcinoma *in situ*, 87% for stage I and 68% for stage II in Japan [1]. Invasive cervical carcinoma is usually treated with radical hysterectomy and bilateral pelvic lymphadenectomy, and adjuvant postoperative radiotherapy is often employed in an effort to improve both local control and survival rate in high risk patients. However, even with optimal surgical results and/or radiation therapy, recurrent carcinoma may develop. Although improvement in local control of pelvic disease in patients with bulky tumours by using combined modality therapy has been reported, there has been no remarkable improvement in overall survival rate because of a corresponding increase in the occurrence of distant metastases [2, 3].

Generally the potency of cellular immunity is decreased in patients with carcinoma. Radiotherapy and chemotherapy give rise to further decreases in immune responsiveness. Numerous

efforts have been made to manipulate immune response mechanisms in attempting to treat various forms of carcinoma to improve survival rate, based upon the suggestion that tumour-associated antigens exist and can induce immune recognition and subsequent reactivity [4].

OK-432, prepared from a penicillin G-treated Su strain of type III, group A *Streptococcus pyogenes*, has been shown to manifest its tumoricidal effect, not only directly, but also by potentiating the host's immune responsiveness [5–7]. The purpose of this study is to determine the effectiveness of OK-432 as adjuvant immunotherapy after radical hysterectomy with or without radiotherapy.

PATIENTS AND METHODS

Patients

From July 1986 to March 1989, 938 cases of uterine cervical carcinoma were recorded and treated by Nagoya Uterine Cancer Study Group, an association comprising Nagoya University and its affiliated hospitals. Patients with adeno- or adenosquamous carcinoma and patients who had undergone an incomplete operation were ineligible as subjects in the study. Of these, 181 patients with stage Ib or II were treated with a radical hysterectomy and bilateral pelvic lymphadenectomy. 86 patients received OK-432 and 95 did not. 4 patients were excluded from analysis because 2 patients had double carcinoma, 1 was more than 70 years of age, and the other had adenocarcinoma. The indications for radiation therapy after complete operation were positive pelvic lymph nodes, positive lymphovascular space involvement, and outer third invasion from mucosa. 177 patients were stratified according to preoperative stage (FIGO classification), radiotherapy, and metastasis of lymph nodes as shown in Fig. 1. Histology and the extent of invasion of all cancers were determined by one pathologist and in 18 patients preoperative staging was not correct. Within each stratum, patients were divided randomly into two groups, OK-432 and control groups, after obtaining written informed consent from each patient.

OK-432

OK-432, a lyophilised powder prepared from a penicillin G-treated Su strain of type III, group A *Streptococcus pyogenes*, was purchased from Chugai Pharmaceutical Co. Ltd., Tokyo. Intradermal administration of OK-432 as a immunotherapeutic agent was started, regardless of radiotherapy, after pathological diagnosis was determined. The starting dose was 1 KE 3 times a week (1 KE corresponds with 0.1 mg dried cells of streptococci), with subsequent gradual increase in the sequence from 1 to 5 KE. The maintenance dosage of the immunotherapy was 5 KE at 2 week intervals for 2 years.

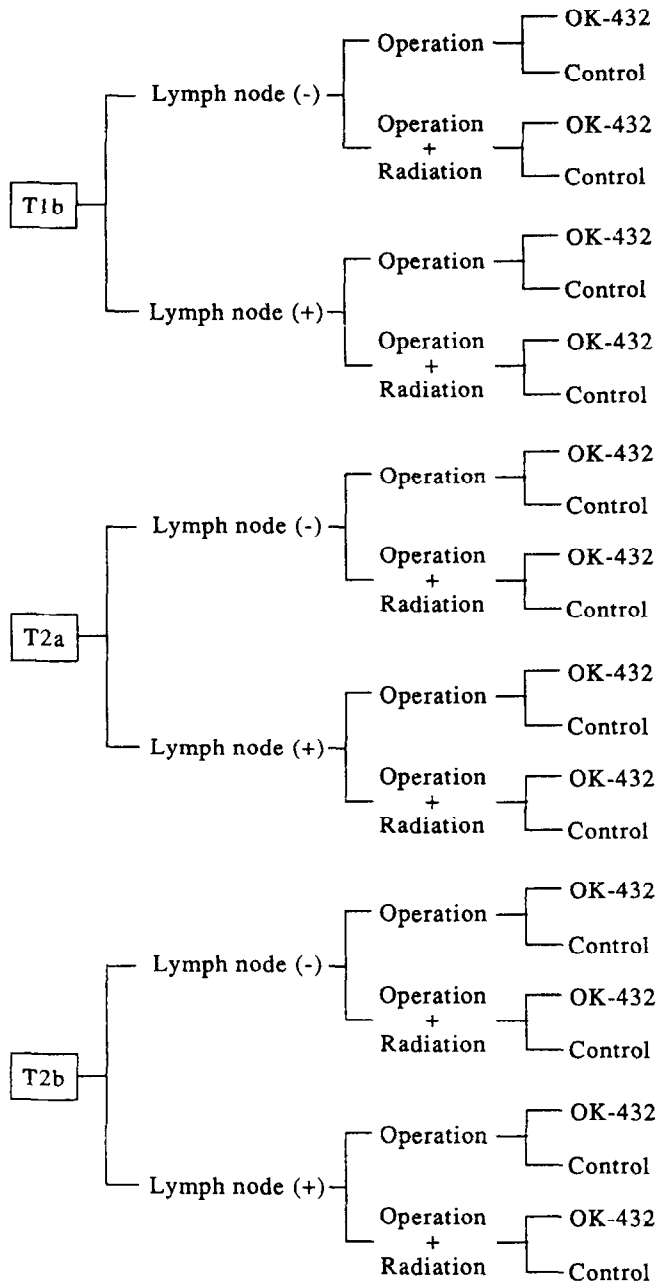

Fig. 1. Stratification and randomisation of patients.

Monitoring patients

All patients had a physical, haematological, and biochemical examination every 2 weeks. Histological examination was carried out every 3 months. Further, all patients had chest X-ray examination, computerised tomography and ultrasonography at 3–6-month intervals.

The Su–polysaccharide (Su–PS) skin test was performed at pre- and postoperative period and every 3 months thereafter to monitor the immunological status of the patient under immunotherapy. The longest and shortest diameters of the erythema were measured 24 h after intradermal injection of Su–PS (20 μg/0.1 ml).

Statistical analysis

The χ^2 test was used to evaluate the background characteristics of patients. The 5-year disease free rate was estimated by

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the Kaplan–Meier method and the statistical significance was evaluated using the log rank, Cox–Mantel, and generalised Wilcoxon procedures.

RESULTS

To minimise the influence of patient background characteristics, eligible patients were assigned to the OK-432 or the control group in each stratum as shown in Fig. 1. Since each stratum consisted of preoperative staging, lymph node metastasis and radiotherapy, postoperative staging and the extent of invasion might possibly have a great influence on disease free rates. Both factors were at essentially the same levels between the OK-432 and the control groups. Moreover, there were no significant differences in distribution by age and histology (Table 1).

No differences were observed between the OK-432 and the control groups in respect of lymphocyte or total leucocyte count (data not shown). SU–PS skin test, however, is the most useful examination of immune responsiveness to OK-432. In the OK-432 group the mean diameter of erythema in the SU–PS test increased rapidly over 6 months and remained so thereafter (Fig. 2). In the control group the mean diameter increased steadily in 24 months, suggesting that the immunological potency was suppressed by the existence of cancer and was recovered by the treatment of carcinoma such as operation and radiation. Significant differences existed between the two groups at 1, 3, 6 and 15 months in this respect.

Figure 3 shows overall disease free survival curves of the OK-432 and the control groups, and indicates that no difference existed between the two groups. In the stratum of stage Ib 42 patients were treated with OK-432 and 48 patients did not receive it (Fig. 4). Five-year disease free rate in the OK-432 group was 89% and in the control group it was 94%. 9 patients (21%) in the OK-432 group and 10 patients (21%) in the control group were noted to have lymph node metastasis. In the stratum of stage II 43 patients were treated with OK-432 and 44 patients were not (Fig. 5). 16 patients (37%) in the OK-432 group and 14 patients (32%) in the control group had lymph node metastasis. All stage II patients with lymph node metastasis were treated by radiotherapy. Five-year disease free rate was 89% in the OK-432 group and 68% in the control group. The difference of 5-year disease free rate between the two groups, 21%, was not significant statistically, however. Figure 6 shows 5-year disease free survival curves in the stratums of stage IIa. There was no

Table 1. Background factors of patients

	OK-432 treatment	Control
Age		
–29	2	2
30–39	17	13
40–49	21	17
50–59	26	30
60–69	19	30
Stage		
Ib	42	48
IIa	18	21
IIb	25	23
Lymph node		
Positive	24	24
Negative	61	68
Histology		
Keratinising	25	41
Non-keratinising		
Large cell	55	42
Small cell	5	9
Invasion		
< 1/3 from mucosa	16	22
< 2/3 from mucosa	13	18
> 2/3 from mucosa	54	51
Not examined	2	1
Radiotherapy		
Linac	68	74
None	17	18
Total	85	92

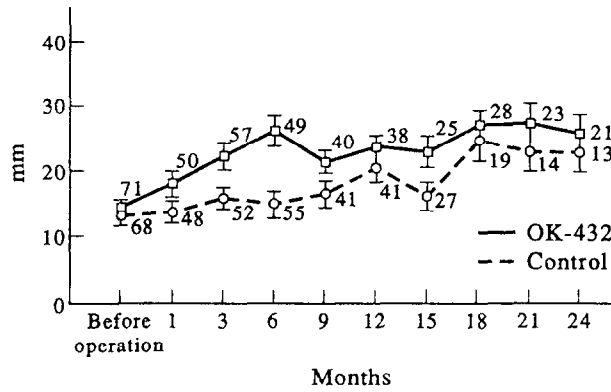

Fig. 2. Changes in the mean diameter of erythema on SU–PS skin test. The longest and shortest diameters of the erythema were measured 24 h after intradermal injection of SU–PS (20 μg/0.1 ml) in the OK-432 group (□) and the control group (○).

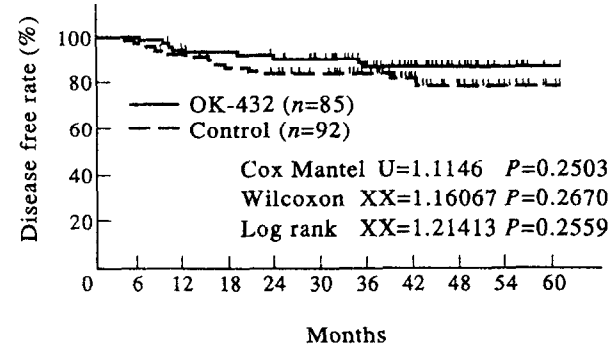

Fig. 3. Overall disease free survival curves in the OK-432 and the control groups.

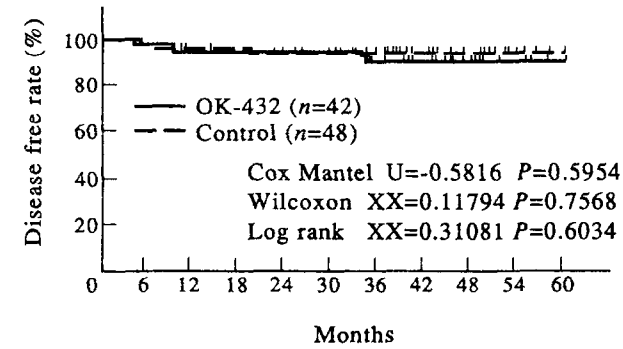

Fig. 4. Disease free survival curves of the OK-432 and the control groups for stage Ib patients.

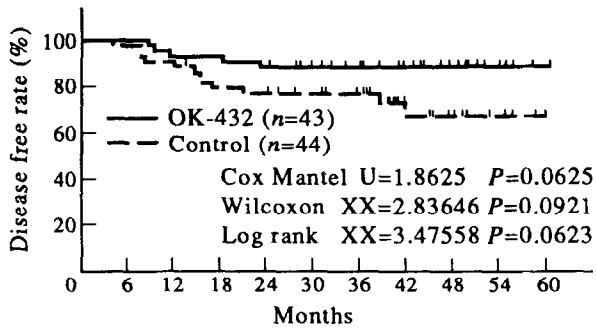

Fig. 5. Disease free survival curves of the OK-432 and the control groups of stage II patients.

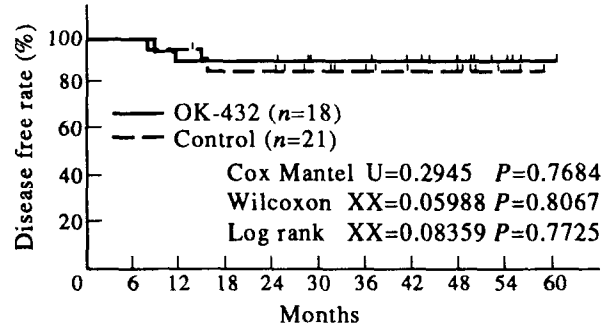

Fig. 6. Disease free survival curves of the OK-432 and the control groups of stage IIa patients.

significant intergroup difference in 5-year disease free survival (90% in the OK-432 group; 85% in the control group). In the stratum of stage IIb, the OK-432 group included 9 patients (36%) with lymph node metastasis and the control group 12 patients (52%) with lymph node metastasis. A significant difference was observed between the two 5-year disease free survival curves (Fig. 7). However, multivariate analysis by means of the Cox proportional hazard model showed that the presence of lymph node metastasis was a significant prognostic factor in stage IIb. The significant difference was not observed by this analysis. Figures 8 and 9 show 5-year disease free survival curves in the stratum of stage II with and without lymph node metastasis, respectively. In the stratum of stage II with lymph node metastasis, the 5-year disease free survival rate was higher in the OK-432 group than the control group. The difference was not significant, probably due to the few patients constituting this stratum. In the stratum of stage II without lymph node metastasis, however, a significant difference was observed

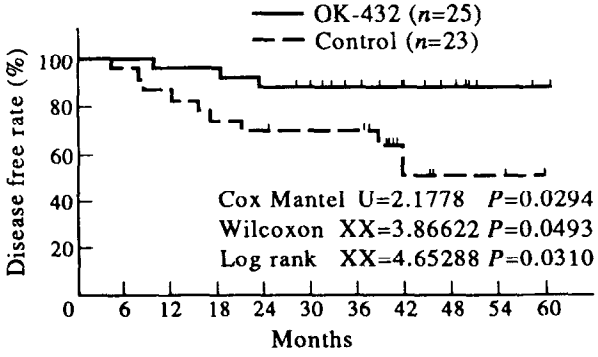

Fig. 7. Disease free survival curves of the OK-432 and the control groups of stage IIb patients.

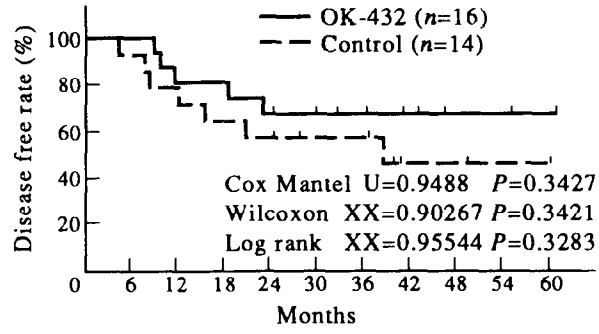

Fig. 8. Disease free survival curves of the OK-432 and the control groups of stage II patients with positive lymph node.

between the OK-432 and the control groups, although the ratio of stage IIb to II was higher in the OK-432 group (58%) than in the control group (52%). All patients who did not receive radiotherapy were alive without disease. Five-year disease free survival rate in the OK-432 group with radiotherapy was 85% and in the control group it was 76% (data not shown). Since radiotherapy was immunosuppressive, OK-432, a immunostimulant, was expected to be effective in patients with radiotherapy. However, there was no significant intergroup difference.

DISCUSSION

With increased patient education and widespread screening programmes, cervical carcinoma is being detected at an earlier stage. Consequently, more patients may be candidates for surgical removal of their tumours. Invasive cervical carcinoma is commonly treated with radical hysterectomy and bilateral pelvic lymphadenectomy. Some of these patients will require postoperative adjuvant radiotherapy due to adverse prognostic factors. Adjuvant radiotherapy enhanced survival in patients with lymph node metastasis [8, 9]. The 5-year survival rate without evidence of disease, however, is 80–90% for stage Ib and 70–80% for stage IIa [10–13]. Since postoperative radiotherapy can control only local disease, it cannot prevent distant metastatic tumours from spreading or progressing. Thus, general treatment is needed to further improve survival.

Biological response modifiers (BRM) may provide an alternative approach to conventional cancer therapy or an adjunct to it [14, 15]. They may augment non-specific antitumor responses of the host by restoring effector mechanisms [16, 17]. Non-specific immunotherapy is now being widely applied in various fields of cancer treatment. OK-432 was first used for injection into the tumour site because of its cytocidal activity exerted by direct contact with tumour cells, i.e. the so-called RNA effect

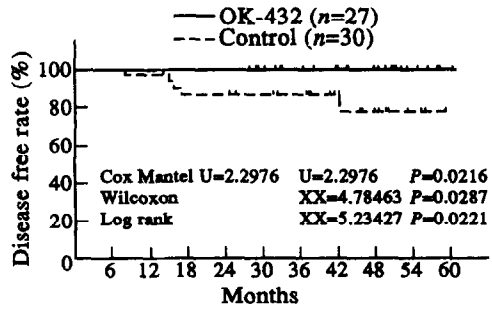

Fig. 9. Disease free survival curves of the OK-432 and the control group of stage II patients without positive lymph node.

[18]. But thereafter, this drug attracted attention because of its host mediated antitumor effect due to stimulation of the immune system of the host [19]. A clinical trial of OK-432 for lung carcinoma demonstrated the effectiveness on survival rate in stage I–IV patients [20]. Fujita reported that the recurrence rates in superficial bladder carcinoma was low in an OK-432 group compared with that in a control group [21]. The Cervical Cancer Immunotherapy Study Group, involving 16 institutions, was established in Japan and reported that the 3-year recurrence free rate in an OK-432 group was significantly higher than that in a control group [22]. Eligible patients were stratified by the presence or absence of surgical operation and clinical stage. Lymph node metastasis is one of the most important risk factors. The 5-year survival rate in stage Ib and IIa patients without lymph node metastasis was 14–40% better than that with lymph node metastasis [23–25]. In the present study, eligible patients were stratified by lymph node metastasis as well as clinical stage and radiotherapy. Since whether the complete operation has been carried out or not is another important factor for recurrence. Patients who underwent complete radical hysterectomy and bilateral pelvic lymphadenectomy were randomly divided into the OK-432 and control groups. OK-432 has been used as a postoperative adjuvant immunotherapy over 2 years, because most recurrences appear within the first 2 years of treatment.

The SU–PS skin test is the most effective examination of the immunological status when OK-432 is used as a immunopotentiating drug, because SU–PS is taken from the cell wall of *Streptococcus pyogenes* [26]. In our data, the mean diameter of erythema was significantly larger in the OK-432 group than that in the control group (Fig. 2). It was reported that systemic administration of OK-432 increased the lymphocyte count and natural killer (NK) cell activity in cancer patients [22]. In our controlled study, however, there were no differences in these two immunological parameters between the OK-432 and the control groups. Nor were there significant differences in the stratum of stage Ib, II, and IIa, although in the stratum of stage II the intergroup difference of 5-year disease free survival rate was 21% (Fig. 5). However, the 5-year disease free survival rate of the OK-432 group in the stratum of stage IIb was statistically better than that of the control group (Fig. 7). Since the 5-year disease free survival rate was about 90%, nevertheless it was difficult to make a clear distinction between the two groups. The significant difference in the stratum of stage IIb may be ascribed to the difference in lymph node metastasis rate, which was 36% in the OK-432 group as against 52% in the control group. In the stratum of stage II with and without positive lymph node, the 5-year disease free rates were higher in the OK-432 group (Figs 7 and 8). Although in the stratum of stage II with negative lymph node metastasis, the OK-432 group included more patients with stage IIb (11 with stage IIa, 16 with stage IIb) than the control group (19 with stage IIa, 11 with stage IIb), a significant difference of 5-year disease free rate was noted. These results have demonstrated that OK-432 can be given safely a dosage levels sufficient to produce an immunomodulating effect, and proved effective at least in stage II patients.

Since the role of BRM in immunotherapy consist in their activities in modulating or enhancing any one of the variety of immune response mechanisms and is supplementary to carcinoma treatment, clinical trials in this area are extremely complex and care must be taken in the design of studies. From the results of our controlled study, OK-432 is worthy of further investigations, which should focus on the optimal administration schedule and the mechanisms to suppress cancer cells *in vivo*.

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