Biological Effect of OK-432 (Picibanil) and Possible Application to Dendritic Cell Therapy

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Abstract. OK-432 (Picibanil), a streptococcal preparation with potent biological response modifying activities, was approved in Japan as an anticancer agent in 1975. In the ensuing 30 years, since then, a significant amount of data, including clinical as well as experimental studies, has been accumulated. OK-432 has been reported to induce various cytokines, activate immunological cells and thus augment anticancer immunity. Recently, the interrelation between innate immunity and adaptive immunity has become clear and it was reported that OK-432 acts, at least in part, via Toll-like receptor (TLR) 4-MD2 signaling pathway. In addition, dendritic cells (DCs) are considered to play a pivotal role in immunological response and it is reported that OK-432 induced maturation of DCs both in vitro and in vivo. These results suggest that OK-432 is a useful adjuvant in DC-based anticancer immunotherapy. Clinical studies of DC therapy with OK-432 are under way.

Dendritic cells (DCs) are professional antigen-presenting cells. The tumor antigen-bearing DCs followed by adequate maturation migrate, present the antigen to T cells, and induce cytotoxic T lymphocytes (CTLs) and helper T cells. On the other hand, Toll-like receptors (TLRs), that are expressed mainly on macrophages and DCs, have been recently identified as molecules that recognize many types of pathogens in addition to host-derived proteins. Macrophages and DCs are not only primarily involved in innate immunity, but are also essential for the establishment of adaptive immunity as antigen-presenting cells. OK-432 is a streptococcal preparation and has already been applied clinically for over 30 years. In this review, we present studies on OK-432, focusing on TLRs and maturation of DCs, and the possible application to dendritic cell therapy, in addition to drug information, clinical results and the biological effects of OK-432.

Development and drug information

OK-432 (Picibanil, Chugai Pharmaceutical Co., Ltd. Tokyo, Japan) is a freeze-dried biological product that is prepared from the Su strain of Streptococcus pyogenes (group A) by treatment with benzylpenicillin and heat (Figure 1). This drug was developed on the basis of studies on streptococci performed by Okamoto and Koshimura (1,2). Heating in the presence of penicillin at 37°C for 20 min and 45°C for 30 min increases the antitumor activity of the Su strain and eliminates its toxin-producing capacity. At the stage prior to freeze-drying, the product is called PC-B-45 (or OK-431) (3,4). Since OK-432 is not subjected to further treatment, such as isolation, extraction or purification, the bacterial cells remain intact (Figure 2). However, proliferative activity is lost and streptococcal infection does not occur when it is administered to humans. Klinische Einheit (KE) is used as a unit of measurement for OK-432 doses. One KE corresponds to 0.1mg of freeze-dried streptococci containing approximately 1 x 10^8 cells (3).

In Japan, OK-432 was approved for manufacture as an anticancer agent by the Ministry of Health and Welfare in 1975. The approved effects and indications currently include: 1) prolongation of survival time in patients with gastric cancer (postoperative cases) or primary lung cancer...
in combination with chemotherapy, 2) reduction of cancerous pleural effusion or ascites in patients with gastrointestinal cancer or lung cancer, 3) head and neck cancer and thyroid cancer that are resistant to other drugs, and 4) lymphangioma (5). OK-432 is administered as systemic injection (intramuscular, subcutaneous or intradermal), as well as intratumor or intraserosal injection for specific cancer. The drug has a history of more than 30 years of clinical use since it was administered to the first patient in 1967. After its approval for lymphangioma, safety information has also been collected in the pediatric field. The main adverse reactions are fever, pain, swelling and redness at the injection site, leukocytosis, thrombocytosis and increased CRP. Since OK-432 contains penicillin, caution is required concerning hypersensitivity (5).

It was first used for lymphangioma in 1986 in Japan (6) and was approved in 1995. Outside Japan, OK-432 has only been approved for marketing in the Republic of Korea and Taiwan ROC, at present; however, the efficacy of OK-432 for lymphangioma has attracted international attention (7). In the USA, a multi-center clinical study based at the University of Iowa is in progress (8). Benefits of OK-432 for cystic disease other than lymphangioma have also been reported (9).

### Clinical results

The reported effects of OK-432 on malignant tumors include control of cancerous pleural effusion and ascites by intraserosal injection (10-13), as well as tumor shrinkage after localized administration into head and neck (14,15) and other cancers (16,17). Prolongation of survival has not been confirmed in independent clinical trials (18-21), however meta-analysis showed a life-prolonging effect in patients with resected non-small cell lung cancer (22) or curative resection of gastric cancer (23). In meta-analysis of 11 randomized studies on patients with resected non-small cell lung cancer (n=1,520), systemic administration of OK-432 showed a significant life-prolonging effect (odds ratio=0.70 (95% CI=0.56 - 0.87) p=0.001) based on the 5-year survival rate as the endpoint (22). Also, meta-analysis of 6 central-enrolment randomized studies on patients with curative resection of gastric cancer (n=1,522) showed that systemic administration of OK-432 had a significant life-prolonging effect (odds ratio=0.81 (95% CI=0.65 - 0.99) p=0.044) based on the 3-year survival rate as the endpoint (23). An individual patient data meta-analysis of the 5-year survival is in progress (24). These results were obtained with injection of the drug, but benefits of the oral administration of OK-432 have also been reported (25).

### Mechanism of action

In the initial stage of development, OK-432 was considered to have actions such as direct inhibition of RNA synthesis in tumor cells (26,27). Other reports of a direct action on tumor cells also appeared, but currently the main action is considered to be via stimulation of host immunity (Figure 3).
In a study using BALB/c mice with cancerous ascites (28,29), induction of various cytokines and the sequential recruitment and activation of neutrophils, macrophages and lymphocytes seemed to be necessary for OK-432 to show an antitumor effect (30). These sequential changes were also clinically observed in carcinomatous ascites (31) and colorectal cancer after local injection of OK-432 into tumor tissue (32).

OK-432 has been reported to induce many cytokines including IL-1 (33-35), IL-2 (33), IFN-γ (35,36,38), TNF-α (34,35,37), IL-6 (39), IL-8 (34), G-CSF (34), GM-CSF (34), IL-12 (35,38) and IL-18 (38). Some of these cytokines act directly on tumor cells to induce apoptosis, changes of cell cycles (40) and enhance the expression of HLA-DR (41), adhesion molecules (ICAM-1) (42) and hormone receptors (43) on tumor cells. IL-12 and IL-18 produced by OK-432-stimulated DCs and macrophages, induce helper T cell 1 (Th1) dominance in the Th1/Th2 balance, and also enhance natural killer (NK) activity (44-48). Furthermore, recent reports demonstrated that OK-432 stimulates to mature DCs and that the OK-432-stimulated DCs can induce tumor-specific CTLs (49-53). Because there are reports on the induction of perforin production (54) and enhanced Fas-ligand expression by OK-432-activated monocytes (55), it appears that an apoptosis-related mechanism is involved in the cytotoxicity of this agent. The cytokine family responsible for hematopoiesis increases the leukocyte and platelet counts by acting on bone marrow cells (56). These cytokines are also involved in the radiation-protective effect of OK-432 (57) and its promotion of liver regeneration (58).

The National Cancer Institute (NCI) has performed preclinical screening of biological response modifiers (BRM) and published the results (59). Compared with other immunotherapy agents, OK-432 was found to have a wide range of actions on the host immune system, including immune cell activation, promotion of cytotoxicity, and inhibition of spontaneous tumor growth in mice (60,61). OK-432 was reported to be significantly superior to BCG (substrain Pasteur) with respect to the induction of IL-12 and IFN-γ (35).

To clarify the mechanism of action of OK-432, research targeting a lipoteichoic acid-related molecule (OK-PSA) on the cell wall is underway (62). OK-PSA was isolated and purified by using a monoclonal antibody that recognizes the molecules related to IFN-γ induction on OK-432. It was confirmed that the OK-PSA content of streptococci is increased by penicillin and heat treatment during the process of OK-432 production (38). In recent years, the importance of innate immunity for establishment of adaptive immunity has been reconsidered and research is being performed on TLRs involved in innate immunity. A study using OK-PSA in mice indicated that OK-432 acts via TLR4. In TLR4-mutant mice (C3H/HeJ), there was no inhibitory effect of OK-PSA on transplanted tumor growth, although this is observed in wild-type C3H/HeN mice (63). In C57BL/6 TLR4-/- mice, the inhibition of transplanted tumor growth by OK-432, which is observed in wild-type mice, was also abolished (64). The relationship between the TLRs and clinical activity was studied in patients with squamous cell carcinoma of the mouth who received combined treatment with OK-432, UFT and radiation (n=28). Among 20 patients with expression of mRNA for TLR4 or MD2 detected by RT-PCR, there were 10 complete response (CR) cases (50%) who did not need surgery because of disappearance of the primary tumor, but no CR cases were found among eight patients without expression of either TLR4 and MD2. Both TLR4 and MD-2 were apparently required for IFN-γ induction by OK-432. No relationship between the effects of OK-432 and TLR2 or TLR9 was found, so the activity of OK-432 appears to involve signaling via TLR4-MD2 (64).
Effects on dendritic cells

The importance of DCs in tumor immunity has been recognized and clinical applications have been investigated. The effects of OK-432 on DCs were studied in detail. It has already been reported that stimulation of IFN production from T cells by OK-432 requires mononuclear antigen-presenting cells (65), that endoscopy intratumor administration of OK-432 significantly increases the invasion of S-100 protein-positive antigen-presenting cells into tumor tissue (66), that antitumor activity was enhanced by intratumoral administration of macrophages with OK-432 (67) and that OK-432 activates murine Langerhans cells in vivo (68). All of these reports suggest that DCs play a pivotal role in the antitumor activity of OK-432.

Several researchers have reported that OK-432 induces the maturation of human DCs in vitro and mice DCs in vivo (Table I). The effects of OK-432 on DCs include: 1) induction of IL-12 and IFN-γ production, 2) enhanced expression of surface antigens (CD40, CD80, CD83, CD86, HLA-DR, etc.), 3) enhanced expression of adhesion molecules (ICAM-1) and 4) induction of peptide-specific CTLs (49-53). It has been reported that it is possible to induce highly reactive DCs in tumor-bearing mice by administration of OK-432 (69). Administration of OK-432 and DCs has been reported to inhibit the growth of transplanted tumors (49,70). In addition, immune cells activated by OK-432 are reported to kill autologous tumor cells (71-73). In patients showing a response of carcinomatous ascites to OK-432, enhanced expression of specific T cell receptors (TCRs) has been observed and cytotoxic activity is inhibited by anti-TCR antibody (74,75). In patients showing a response to OK-432, not only non-specific immunity (such as NK cells), but also tumor-specific reactions are induced, and it is suggested that DCs are related to the induction of specific immunity. Clinical studies on the concomitant use of OK-432 and DCs have been started (76).

Conclusion

Basic studies performed in recent years have suggested that signaling via TLR4 has an important role in the actions of OK-432 and that OK-432 can promote the maturation of DCs. Although this article has mainly concentrated on DC-related reports, concomitant application of OK-432 and a tumor cell vaccine with the GM-CSF gene has also been studied (77) and enhancement of antibody-dependent cellular cytotoxicity (ADCC) activity by OK-432 using a monoclonal antibody has been reported (78-80). Clinical use of antibody is generalized at present. In the near future, the clinical application of new specific immunotherapies, such as vaccines and cell therapies should expand. OK-432 has wide-ranging effects on immune cells and has already been applied clinically for over 30 years. Although it is a well-established drug, it is one of the agents showing the most promise for clinical application as an adjuvant to enhance the effects of specific immunotherapy.

References


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