CHAPTER 27

MEDICAL ASPECTS OF LENTINAN ISOLATED FROM *LENTINUS EDODES* (BERK.) SING.

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1. INTRODUCTION

The most important aim of cancer research is to increase the survival time of cancer patients, keeping their lives comfortable and, if possible, achieve a complete cure of the cancer itself. However, cytocidal anticancer chemotherapeutics generally exhibit severely detrimental side effects, and markedly suppress the host defences against cancer. This hardly meets the purpose of extending the patients survival time except for some specific cancers.

Therefore, new wine is necessary for a new wineskin; i.e. totally new ideas are indispensable for the development of new anticancer drugs. How can these drugs be developed and how can they be used most successfully? This article concerns the evaluation of lentinans as Host Defence Potentiators (HDPs), their mode of action, and their experimental and clinical application to cancer and infectious diseases.

2. LENTINANS AS HOST DEFENCE POTENTIATORS (HDPS)

In studies on the development of anticancer drugs, the concept of killing cancer cells is basically wrong. We should be interested not in such cytocidal chemotherapeutics or immunotherapeutics, but in the augmentation of intrinsic host defence mechanisms against cancer and infectious diseases in the human body, and in the discovery of substances which can enhance this host defence, namely HDPs. This issue concerns the host-tumour relationship in the human body. The most important problem is to understand how the cancer and host compete against each other. When the cancer wins the host dies, and when the host wins the cancer is cured. It is generally accepted that the proliferation and spontaneous regression of tumour cells are balanced at the level of 10^7 cells in immunized mice. If the threshold of the balance is raised up to the level of 10^9, 10^{10} cells or more by augmenting the host defence mechanisms, this will be beneficial for cancer patients. The drugs facilitating this effect should be called HDPs.

The existence of host defence mechanisms against cancer is reflected particularly in the prognosis of patients after surgery. Their existence is also indicated by the spontaneous regression of cancer, or by recurrence even 10-50 years after curative operations on cancer patients. In the latter situation, it is assumed that cancer and host have coexisted for a long time. The most important objective in cancer therapy is, therefore, to markedly augment the host defence activity of patients in every respect. Lentinan is a substances which can improve the physiological constitution of the host against cancer and against various kinds of infections. Thus, it can be said that lentinan can restore or augment the ability of the host to respond to bioactive substances such as lymphokines or cytokines by stimulating maturation, differentiation or proliferation of the cells important for host defence mechanisms.

Agents such as interleukin-2(IL-2) or tumour necrosis factor (TNF) are not categorized as HDPs. Their action is generally localized, not particularly selective, and their half-life in the human body is short. Systemic administration of these agents at large dosage levels is rather against the maintenance of homeostasis of the host and the results are not so different from those following administration of ordinary anticancer chemotherapeutics. Their effectiveness would, therefore, be limited to local administration. These agents appear to have developed from the idea of killing tumour cells and are completely different in nature from lentinan.

3. BIOLOGICAL ACTIVITY OF LENTINAN

Lentinan appears to represent a unique class of HDPs potentiating the physiological constitution of the host against cancer and infectious diseases. This substance was discovered initially in a hot water extract of *Lentinus edodes* (Berk.) Sing., an edible mushroom, and completely inhibited the growth of sarcoma 180 implanted subcutaneously in ICR mice (Chihara *et al.*, 1969). From the extract, we isolated and purified a polysaccharide which showed marked antitumour activity, and named it lentinan.

Lentinan is now proven to exhibit prominent antitumour activity not only in allogeneic, such as sarcoma 180, but also syngeneic and autochthonous hosts without any noticeable side-effects. Lentinan markedly prevents chemical and viral oncogeneses (Suga *et al.*, 1984), and suppresses cancer metastasis and recurrence in animal models (Suga *et al.*, 1989). Therefore, lentinan is not merely a stimulant of homograft rejection but a true antitumour substance.

Furthermore, lentinan was demonstrated to enhance host resistance against infections from various types of bacteria, viruses, fungi and parasites (Chihara, 1990). It suppressed post-chemotherapy relapse in experimental mouse tuberculosis, and proved to be effective against infections from VSV-encephalitis and Abelson virus, *Schistosoma mansoni* and *S. japonicum*. Recently, it has been reported that lentinan and some of its derivatives are effective against HIV-1 or AIDS infections.

4. MECHANISM OF ACTION OF LENTINAN

Lentinan has no direct cytotoxicity to target cells and its action is host-mediated. Why are lentinans referred to as a unique class of HDPs potentiating the physiological constitution of the host against cancer? It is because lentinan is able to augment the host's response through maturation, differentiation and proliferation of lymphoid cells and other physiologically important cells active

in host defence mechanisms.

Lentinan is defined as a T-cell oriented adjuvant, in which macrophages play some part, which displays various kinds of immune reaction in normal and tumour-bearing hosts (Maeda & Chihara, 1971). However, it has not been clear how lentinan affects the host at an early stage, prior to the induction of many immune reactions. We have observed the appearance of various kinds of bioactive serum factors immediately after the administration of lentinan (Maeda *et al.*, 1974). Most of them were induced by macrophages, i.e. acute-phase protein inducing factor (APPIF), vascular dilatation and hemorrhage inducing factor (VDHIF), IL-1 production inducing factor (IL-1PF), interleukin-3 (IL-3) and colony stimulating factor (CSF), which peaked several hours after the administration of lentinan. These bioactive serum factors appear to act on lymphocytes, hepatocytes, vascular endothelial cells or synovial fibrobrasts to cause many host defence reactions associated with inflammation and immunity (Maeda *et al.*, 1984). Moreover, lentinan activates the classical and alternative pathways of the complement system resulting in augmentation of non-specific cytotoxic activity of macrophages. Infiltration of neutrophils into tumour modules should also be noted.

The induction of a remarkable increase in the amount of IL-1, as well as of CSF and IL-3, is also a most important activity of lentinan. This leads to the maturation, differentiation and proliferation of immunologically important cells of the host defence mechanisms such as premature lymphocytes. This, in turn, results in a significant enhancement of the host's capacity to respond to lymphokines and cytokines such as IL-2, and to many other physiologically active substances. Although lentinan does not specifically accelerate the production of IL-2 from helper T-cells, it potentiates the induction of different types of antitumour effector cells, such as killer T-cells, NK-cells and cytotoxic macrophages, as a result of sequential reactions. Under these circumstances, the effector cells may act either selectively or non-selectively on target cells; therefore, it may be wrong to classify lentinan as a non-specific immuno-stimulant. An *in vitro* incubation of thymocytes (immature precursor T-cells) of mice administered a very small amount of lentinan (0.001mg/kg) with IL-2 leads to the production of many allo-killer cells killing EL-4 lymphoma. This result indicates that thymocytes of mice administered lentinan have been enhanced in terms of their responding capacity to IL-2. In other experiments, lentinan did not activate NK cells *in vitro* whereas poly I:C, an interferon inducer, did. However, this experiment showed that, on the basis of enhanced cytotoxicity to YAC tumour cells, NK activity induced by poly I:C *in vitro* was much higher when spleen cells of mice administered lentinan were used instead of normal spleen cells. The same results were obtained using IL-2 instead of poly I:C. Since lentinan did not induce interferon or activate NK cells *in vitro*, these results suggest that, *in vitro*, lentinan markedly enhances the responding capacity of NK cells to IL-2 or NK-activating factors. These are examples showing the potentiation or restoration of the physiological antitumour constitution of the host. In other words, maturation and differentiation of important cells for host defence are essential, which is the most important principle of host defence potentiators (Hamuro & Chihara, 1985).

5. CLINICAL APPLICATIONS OF LENTINAN

Clinical applications of lentinan were examined as a result of the marked antitumour activity, negligible side-effects and the HDP mode of action in experimental studies. Lentinan is worthy of consideration as an effective therapy for cancer patients.

The results of a five year follow-up study on lentinan for advanced and recurrent gastroinstestinal cancer in Phase III have proved to be excellent: a combination therapy of lentinan with tegafur

produced a marked increase in the survival time of patients than tegafur alone. In the case of stomach cancer, the survival rates of patients of one, two and three years after the administration of tegafur alone showed increases of 2.9%, 2.9% and 0% Patients who had been treated with tegafur in combination with lentinan, 1mg twice a week or 2mg once a week administered intravenously, showed survival rate increases of 19.5%, 10.4% and 6.5%, respectively (Taguchi, 1987).

Similar results were obtained for advanced and recurrent colorectal cancer. In these cases, the 50% survival time of patients who had been treated with tegafur alone was 94 days, whereas it increased up to 200 days in the group of patients treated with tegafur plus lentinan. The results were rather disappointing when lentinan was combined with mitomycin C and 5-FU, both of which are more strong anticancer chemotherapeutic agents than tegafur. This finding suggests that lentinan may be more effective when used as a single agent rather than in combination with chemotherapeutic agents. Chemothepeutics may be beneficial when administered once to several times in cases of absolute necessity but not for long term administration. The results for patients with esophageal cancer, squamous cell lung cancer and breast cancer were found to be excellent in combination therapy involving surgery, radiation and lentian therapy without chemotherapy.

6. FUTURE PROSPECTS OF LENTINAN

Suppression of cancer recurrence after surgical resection is another most challenging goal for lentinan therapy. From the above mentioned clinical results and mode of action against tumour cells, lentinan would be expected to bring much better results when used for preventing the recurrence of cancer and the onset of AIDS symptoms in HIV carriers. At present, the five year survival ratio of lung cancer patients after resection is only 51.1% at the National Cancer Centre Hospital, Tokyo, even in cases without any lymphonode metastasis (Watanabe & Suemasu, 1982). Tumour cells are hidden somewhere within the body for the other 48.9% of patients. Even if recurrence is suppressed in 10% or so of these patients, an extremely large number of patients would be affected worldwide. The use of lentinan would be most appropriate and most reasonable for this purpose. In this context, adjuvant chemotherapy using conventional anticancer chemotherapeutics is generally not so effective, and may be harmful by accelerating the formation of metastasis through immunosuppression of the host. This can be realized not by direct attack of cancer cells but by suppression of the proliferation of small numbers of autochthonous cancer cells scattered throughout the body of the host. The best way to attain this may be to potentiate host resistance against cancer so as to suppress proliferation of the cancer cells. There are no magic anti-cancer drugs that are capable of eradicating a large mass of cancer all at once.

There are several reports showing that recurrence after surgery was suppressed by lentinan in animal models. Using MH-134 hepatoma cells, lentinan was shown to completely prevent recurrence of cancer after surgical operation. A similar result was reported using Madison-109 lung carcinoma, DBA/2.MC.CS-1 and DBA/2.MC.CS-T fibrosarcoma. Clinical studies on the prevention of recurrence of many kinds of cancer after surgery are now underway.

The same principle is applicable to AIDS therapy: prevention of clinical manifestation of AIDS symptoms in HIV-1 carriers is most important and this can be realized by HDPs such as lentinan. Recently, our colleagues have reported that lentinan and some of its derivatives are effective against AIDS (Kaneko & Chihara, 1992). Lentinan reduced the toxicity of AZT, and sulphated lentinan inhibited reverse transcriptase activity more strongly than dextran sulphate and proved to be effective against HIV virus. Prevention of the onset of AIDS symptoms through potentiation of host defence

is now being actively investigated experimentally and clinically.

7. CONCLUSION

Any of the physiologically active substances, including lympho-cytokines hormones and neurotransmitters become useful only when the host response to these bioactive substances is fully restored to, or augmented above, the normal state. Lentinan is a substance that potentiates this "physiological constitution of the host". This is particularly important in cancer patients in an immunosupressed state. Agents such as lentinan produce a good response not only to cancer but also to infectious diseases, and might also be associated with protection from aging. The leading principle of the function of lentinan resides in the fact that it can cure patients by restoring their homeostasis, and through enhancement of their intrinsic resistance against such diseases. This implies restoration to a normal from an abnormal state in a relevant area of immunology, endocrinology, neurology or nutrition. Potentiating the physiological constitution in favour of the host defences results in the activation of many kinds of cells that are vitally important for the maintenance of homeostasis. New and widely varying host defence potentiators, having novel characteristics and associated specifically with nervous, endocrine and immune systems, should be further developed. These concepts may also play an important role as a bridge between modern immunology, biology and oriental medicine (Chihara, 1992).

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