The Antitumor Activity of the DNA Fraction from Mycobacterium bovis BCG (MY-1) for Glioblastoma

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(Received 9 December 1994/Accepted 22 February 1995)

ABSTRACT. The antitumor activity of the DNA fraction extracted from Mycobacterium bovis BCG (MY-1) for glioblastoma was investigated in the experimentally produced brain tumor in rats. The tumor-bearing rats were given intrasalosal injection of 1 mg of MY-1 twice a week for three weeks, and were sacrificed for comparison with those of control rats. The main macroscopic features of the tumors treated with serial injections of MY-1 were cystic and destructive structures, which were histologically characterized by multiple microcysts containing macrophages. Furthermore, infiltration of leukocytes as well as the perivascular cuffing in the marginal area was observed. These findings suggested that the serial injections of MY-1 into the brain tumor have the therapeutic potential for glioblastoma.—KEY WORDS: glioblastoma, immunotherapy, MY-1.


Recently many biological response modifiers have been used as an adjunctive cancer therapy, and the extract from Mycobacterium bovis BCG is one being proved for its antitumor activity against some syngenic experimental tumors [3-6, 9, 10]. The extract named MY-1, with a characteristics feature of high DNA content up to 70%, is expected for its extensive antitumor activity [3, 10].

The systemic administration of MY-1 into the tumor-bearing animals has reported to be effective and their tumors decreased in size [5]. The activation of the host immune system was considered to be involved in its antitumor activity, with no direct cytotoxicity to tumor cells [3-6, 9, 10]. MY-1 was also proved to be most effective when administered topically into the tumor tissue. This activity of MY-1 was reported to be dose-dependent [5]. These results indicated that an intrasalosal injection of MY-1 may have the therapeutic potential to the malignant tumor as a new biological response modifier.

Glioblastoma is one of the typical malignant brain tumors. The clinical applications of several biological response modifiers on glioblastoma have been performed previously with unstable satisfactory results. The antitumor effect of MY-1 has been mostly recognized in cutaneous tumors, and has never been studied for its therapeutic potential against glioblastoma.

It is generally recognized that the intrasalosal injections of some biological response modifiers into the brain tumor could achieve the high concentration of the drugs in the tumor and peritumoral tissue regardless of blood-brain barrier [7, 8]. This study was, therefore, conducted to investigate the effect of the intrasalosal administration of MY-1 on the experimentally produced brain tumor in rats with major interest in macroscopic and histopathological changes of the tumor.

Three-week-old male Wistar rats (Japan SCL, Shizuoka, Japan) were fed ad lib. and had free access to the water throughout the experiment. Brain tumor was produced by intracranial inoculation of C6 cells (5.0 × 10⁶ /head) into nucleus caudate in right hemisphere of the brain [1, 2]. MY-1 was obtained in freeze-dried form (Mitsui Pharmaceuticals, Inc., Tokyo, Japan). Three weeks after the inoculation of the tumor cells, twenty tumor-bearing rats were randomly divided into two groups of ten rats each, one for MY-1 treatment and another for control.

Under general anesthesia with pentobarbital given intraperitoneally (40 mg/ kg), rats were fixed to the stereotaxic frame, and a midline incision of the head skin was made. In the MY-1 treated group, 1 mg of MY-1 dissolved in 25 μL PBS was injected directly into the brain tumor using microsyringe through the burr hole made on the skull. The burr hole was covered with bone wax and the skin was closed with a surgical clip. In the control group, 25 μL of PBS was injected. The same procedures were repeated for all rats twice a week for three weeks successively.

The rats were sacrificed under the pentobarbital anesthesia by the whole-body transcardiac perfusion with 10% formalin after the initial perfusion of heparinized saline solution. The brain was taken out of the skull three days after the last injection and was subjected to gross observation being followed by the routine histopathological examination.

During the serial injections of MY-1 or PBS into the brain tumor, three tumor-bearing rats in MY-1 treated group and five rats in control group had died due to the extensive growth of the intracranial tumor, which was confirmed at the time of necropsy. The tumors were confirmed as glioblastoma. No harmful effects of MY-1 for the rats could be observed throughout the experiment. There was no significant difference in survivals between MY-1 treated and control group.

The typical macroscopic findings of the brain tumors in MY-1 treated group and those in control group were shown in Fig. 1. The brain tumor received MY-1 showed the cystic and destructive structures, which were observed widely throughout the tumor occupying region. On the other hand, the tumor received only PBS occupied the most of the right hemisphere of the brain and seemed to be viable. Compression of the ventricles and the midline shift in the MY-1 treated group were less severe compared to those in control group, suggesting that increased intracranial pressure due to the growth of the tumor was markedly improved after the serial injections of MY-1. However, the neurological disturbances of the tumor-bearing rats could not improve during the serial injections.
Fig. 1. Typical macroscopic findings of the serial sections of the experimental brain tumor received 1 mg of MY-1 for six times (A-C) and those in the control group (D-F). MY-1 treated tumor (A-C) exhibits the destructive structures of the tumor tissue, whereas the control tumor seems to be viable and occupied large part of the right hemisphere of the brain.

Fig. 2. Microscopic findings of the tumor treated with MY-1: Microcysts containing some macrophages (A) and degenerative changes of the tumor cells (B) are observed. HE, A: \( \times 200 \), B: \( \times 400 \).

Fig. 3. Extensive leukocyte infiltration in the MY-1 treated tumor (A) and perivascular cuffing in the marginal area (B). HE, A: \( \times 400 \), B: \( \times 200 \).

of MY-1.

In the histopathological study on MY-1 treated tumors, multiple microcysts containing large number of macrophages were observed and were the most obvious findings. The nuclei of the tumor cells in the microcysts were shrinking, and karyolysis was found occasionally (Fig. 2). No viable tumor cells could be observed in these destructive regions. In addition to this, the massive accumulation of mononuclear leukocytes as well as the perivascular cuffing in the marginal area were also seen in some treated tumors (Fig. 3). The direct cytotoxicity of MY-1 for various tumor cells [5], including C6 cells, has not been observed. These results may indicate that the immune system of the host was activated by the serial injections of MY-1 and the tumor tissue regressed owing to the effects of infiltrating immune-mediated cells, even in the tumors originated from central nervous system.

Activated macrophages are realized to be major effector cells in the antitumor effects of BCG [11]. Macrophage is also required for the activation of other effector cells involved in the antitumor activity of MY-1 [6]. We were also able to observe the degenerative tumor tissue including many macrophages. Accordingly, our findings may strongly suggest the possibility for activated macrophages to play some significant roles even in the regression of glioblastoma as in the cutaneous tumors.
Furthermore, the infiltration of mononuclear leukocytes was also observed in the brain tumors treated with MY-1. Kuramoto et al. reported the infiltration of mononuclear cells positive for asialo-GM1 in the skin tissue injected with MY-1 [4]. These cells are considered to be natural killer cells and are also believed to be the most important effector cells in the antitumor activity of MY-1 [6, 10]. However, we did not investigate the surface makers of these infiltrating mononuclear cells. For more conclusive identification of these leukocytes observed in this study, further investigations must be needed.

In the normal brain tissue surrounding the tumor, the infiltrating tumor cells were found regardless of the degeneration of the tumor tissues. Glioblastoma is known to be extensive in the infiltration, generally resulted in guarded prognosis. The adjunctive cancer therapy must be intended to damage even these infiltrating tumor cells to prevent tumor recurrence. From our results, MY-1 used alone for glioblastoma could not be effective in damaging these tumor cells.

In conclusion, the results of this study are suggestive enough that intratumoral serial injections of MY-1 are a useful immunotherapeutic measure against the glioblastoma, but the complete remission could not be induced by MY-1 alone. Other therapeutic modalities must be used in combination with MY-1.

REFERENCES