The growth and spontaneous dissemination of melanoma B16 and Lewis lung tumour in two sub-strains of C57BL/6J mice treated with Corynebacterium parvum and/or levamisole

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(Received September 1982; accepted January 1983)

The effect of C. parvum and/or levamisole on tumour growth and spontaneous dissemination was tested in two tumour-host systems, melanoma B16 and LLT in C57BL/6J mice. C. parvum inhibited the growth of LLT and its dissemination to the lungs and the growth of B16 in females in one of the two sub-strains of mice used. Levamisole stimulated the growth of B16 in females in one sub-strain and in both sexes in the other sub-strain of mice. The growth of LLT was not influenced by levamisole but its dissemination to lymph nodes was facilitated. The growth-stimulating effect of levamisole was abrogated by C. parvum in the mice treated with both agents in combination. It is concluded that the effects of non-specific immunomodulators on tumour growth and spread can vary between sub-strains of the same inbred strain of mice bred at different laboratories.

Introduction

Corynebacterium parvum (C. parvum) has been found to be a potent stimulator of the reticuloendothelial system (RES) [7] and an antitumour agent [8, 23]. Its tumour-suppressive effect has been documented in several tumour-host systems. Intra-tumour injection is the most effective route of administration, causing complete tumour regression [12, 13, 22, 24], while the effect of systemic administration varies with the tumour-host system and with the timing of the injection [1, 4, 14].

Levamisole, a potent anthelmintic drug [21] and a nonspecific stimulator of the immune system [10], was also found to inhibit the growth and spread of Lewis lung tumour (LLT) [17]. However, the tumour-suppressive effect of levamisole could not be confirmed in the same system by Johnson et al. [11] or in other systems by Johnson et al. [11] or Hopper et al. [9]. The combination of the two nonspecific immune-modulators C. parvum and levamisole was, however, more effective against two mice tumours, LLT and P815 mastocytoma, than either drug alone [1].

There is increasing experimental evidence of an antitumour and antimetastatic effect of C. parvum although these data suffer from lack of standardization of the batch, dose and route of administration of C. parvum. The Metastasis Project Group of the European Organization for Research on Treatment of Cancer (EORTC) created a cooperative study aimed at investigating the effect of C. parvum in combination with other drugs. The present work, in which C. parvum is combined with levamisole, is part of this cooperative study. The batch of C. parvum, its dose and the mode of administration were standardized and the tumours used were LLT and melanoma B16.
Material and methods

Melanoma B16 and LLT were transplanted into inbred C57BL/6J mice aged 5–7 months. The C57 mice originated from Bomholtgård (Bom) Denmark or were bred in our own laboratory (Lkpg). Solid pieces of the tumour were transplanted subcutaneously into the tail according to the method of Hagmar and Boeryd [6]. Each experimental group comprised 10 mice (males and/or females).

The mice were treated before or after tumour amputation, i.e. *C. parvum* was given on day 15 and levimazole on day 16 after tumour transplantation of 4 and 5 days respectively after tumour amputation (see tables). The effects of *C. parvum* and/or levimazole on the growth and spread of B16 were tested in three experiments. In two of these, one with C57/Lkpg mice and one with C57/Bom mice, the treatment was given before tumour amputation. In the third experiment, with C57/Bom mice, the treatment was given after tumour amputation. In one experiment with LLT the C57/Bom mice were treated before amputation.

*C. parvum* (batch ca 732 from Wellcome Research Laboratories, Beckenham, England) was given i.v. in two different doses, 0.35 or 0.70 mg. Levimazole was given i.p. in a dose of 3 mg/kg body-weight. In some groups the two drugs were combined. Because of the faster tumour growth in females, the tails with the tumours in this sex were amputated some days earlier than in males (see tables). Tumours were weighed with 2 cm of the tail. The experiments were terminated when a few mice had died of metastases.

An autopsy macroscopic extrapulmonary metastases were noted. The lungs from mice transplanted with B16 were prepared for histological investigation as previously described [2]. Lung metastases from LLT were so numerous that the weight of the lungs was used as a measure of the metastasis. In the few mice without macroscopic pulmonary metastases the lungs were sectioned for histological investigation.

Statistical methods

Differences in weight of the tail tumours and weight of the lungs were tested with Wilcoxon's two sample rank test. Differences in incidence of metastasis were analysed according to the fourfold table test.

Results

*Melanoma B16*

In C57BL/6J/Lkpg mice levimazole given before tumour amputation facilitated the growth of B16 in females (*p* < 0.05) while the incidence of metastasis was not significantly increased. Neither of the two doses of *C. parvum* affected tumour growth or the incidence of metastasis. In mice treated with the two drugs together, *C. parvum* counteracted the stimulating effect on tumour growth of levimazole alone in females (table 1).

In C57BL/6J/Bom mice, levimazole given before amputation stimulated the growth of B16 in both sexes (*p* < 0.05). *C. parvum* alone in a dose of 0.35 mg inhibited tumour growth in females (*p* < 0.05). In mice treated with levimazole and *C. parvum* tumour growth was inhibited in females (*p* < 0.05). In males the tumour weight was less than in mice treated with levimazole alone although still higher than in controls (*p* < 0.05). The incidence of metastasis was not significantly influence by any treatment (table 2).