METHIONINE ENKEPHALIN

A New Cytokine with Antiviral and Antitumor Activities

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Recently met-enkephalin has been identified to be derived from its prohormone, pro-enkephalin A in macrophages and T helper cells. Its immunological profile is similar to IL2 and gamma interferon. Met-enkephalin specifically increases numbers of cytotoxic cells (CD8 and NK) that are effective against viruses and tumors. Anti-viral activity was demonstrated against Herpes, AIDS, CMV, coronavirus, FluA, and Japanese Encephalitis either alone or in combination with known anti-virals. Anti-tumor activity was seen against melanoma, sarcoma, lung cancer, colon, leukemia, and neuroblastoma. In addition, clinical studies in AIDS and cancer patients are discussed.

Mind-body relationships probably involve interactions between hormones, neurotransmitters, cytokines and many other factors. Methionine enkephalin, which was originally identified to be an opioid peptide, neurotransmitter and hormone was more recently identified also to be a cytokine of the immune system (1) (2). The immunological effects of methionine enkephalin have been extensively reviewed and include increases of T cell subsets, NK cells, as well as macrophage activation (3). These effects were particularly striking in the treatment of AIDS and cancer patients. The presentation today, will focus on the broad spectrum of antiviral and antitumor activities of methionine enkephalin.

ANTIVIRAL ACTIVITIES OF METHIONINE ENKEPHALIN (MET ENKEPHALIN)

Influenza A Virus

Methionine enkephalin was found to increase natural killer and cytotoxic T lymphocyte activity in mice infected with influenza A/NNS/33 (H1 N1). Maximal increases in cytotoxic cells were seen when met-enkephalin was administered 72 hours after viral infection (4). It is interesting to speculate whether met-enkephalin enhances other cytoki-

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nes, that are released from activated lymphocytes. Thus, met-enkephalin has been found to increase interleukin II levels and expression of receptors (5). This study provides a basis for considering treating hospitalized patients with "viral pneumonia". In a pilot study, AIDS patients with pneumonitis, were successfully treated with met-enkephalin (6). Since diagnosis of hospital viral pneumonias is difficult, it would not be unreasonable to consider combining met-enkephalin with antibiotic therapy. Precedence for this clinical approach was established with the cytokine, G-CSF and antibiotics in the treatment of immunosuppressed cancer patients (7).

Japanese and Tick-Borne Encephalitis

Peptides, derived from bone marrow, were found to have therapeutic effects against Japanese and tick-borne encephalitis. The principle components of these peptides were found to be enkephalins with opioid binding properties and were named myelopeptides. These peptides were found to stimulate anti-viral antibody production (2 to 5 fold) against Japanese encephalitis virus and also the langat virus of the tick-borne encephalitis TP-21 complex. Treatment with the peptides doubled the survival rate. These protective effects were seen both in acute and chronic infections. The maximal effects on antibody production was seen in activated cells at the peak of the immune response (8). This enhancement of activated cells suggests that there may be synergism with other cytokines such as interleukin II and gamma interferon (5)(9). No toxicity was observed with the use of these peptides.

Rauscher Virus (RLV)

AZT has shown potent activity against Rauscher murine leukemia virus complex (RLV) in vitro and in vivo. The pathology of RLV in BALB/c mice involves massive splenomegaly in two to three weeks post-inoculation (plus an increase in serum virus titer and serum reverse transcriptase activity). "Met-enkephalin prevented splenomegaly and reverse transcriptase activity slightly, but not significantly, at both 1.2 and 3.4 mg/kg/day doses. Also, the addition of 1.2 mg/kg/day of Met-enkephalin to an oral dose of 1.6 mg/kg/day of AZT produced a statistically significant prevention of splenomegaly and reverse transcriptase activity compared to untreated controls, while an oral dose of 1.6 mg/kg/day of AZT by itself did not". Combination therapy of antivirals and cytokines is now believed to be the best approach for the treatment of HIV (10).

Friend Leukemia Virus (FV) and Maids Virus (BM5)

FV induces a severe immunodeficiency and leukemia in susceptible BALB/c mice. AZT (azidothymidine) reduces morbidity and lengthens survival of infected mice. Combination of AZT and met-enkephalin decreases morbidity and increases survival beyond that seen with AZT alone. Both agents were administered to infected mice beginning 3 days p.i. and administered over four weeks. All mice surviving at 70 days p.i. were killed and their spleen weighed. Approximately fifty percent (14/30) of the mice survived at 70 days using the combination of AZT and met-enkephalin (11). Ciolli et al reported that administration of IL-1 and IL-2 resulted in increased survival of infected mice (12). Since met-enkephalin has been reported to increase IL-1 and IL-2 production, it is possible that the three cytokines may be acting together synergistically in protecting FV infected mice (13) (5). In the case of the MAIDS virus, AZT/met-enkephalin combination markedly reduced splenomegaly.

Herpes Simplex Virus Type 2

Methionine-enkephalin significantly increased the short-term survival of mice after they were challenged with an LD80 dose of HSV-2. The treatment was conducted over a period of 24 days. In a separate study met-enkephalin was added to culture media of VERO cells infected with HSV-2. No direct antiviral effects were seen. However, met-enkephalin was demonstrated to significantly increase NK activity of splenic lymphocytes (14). In addition, clinical studies in AIDS patients showed that met-enkephalin treatment markedly reduced herpes lesions (15).

Coronavirus MHV-JHM

The neurotropic strain of the mouse hepatitis virus produces encephalitis and a paralytic-demyelinating disease in rodents. It has been used as a model of multiple selenosis. B-endorphin was found to reduce the paralytic demyelinating disease in mice by 40–50%, compared to controls. Virus replication in the brain was significantly reduced 3 days post-infection (16).

Cytomegalovirus (CMV)

Only slight protection was seen against murine CMV when mice were treated with ganciclovir plus met-enkephalin (at low threshold doses). The combination treatment appeared to diminish mortality and increase the median day of death (17).

AIDS Patients

In a serious of clinical studies in ARC and AIDS patients met-enkephalin treatment was found to increase T cell subsets (CD3, CD4, and CD8), NK cells, IL2, and blastogenic responses to PHA (3). Also, met-enkephalin and reduced viral load, as measured by p24 levels (15). Finally, met-enkephalin increased cell-mediated immunity (via-THI cells) as well as IL2 and gamma interferon production (18). Combination studies of met-enkephalin and the new anti-virals are currently in progress.

ANTITUMOR STUDIES OF METHIONINE ENKEPHALIN

Human Colon Cancer

Methionine enkephalin at dosages of 0.5, 5.0, or 25mg/kg diminished the development of human colon cancer HT-29 xenografts in nude mice. Seven weeks after cancer cell inoculation, 57% of the mice treated with methionine enkephalin did not develop a tumor. A new opioid receptor (zeta) is believed to regulate oncogenesis with met-enkephalin being the specific ligand (19).

Human Lung Cancer Cell Lines

Opioid receptors have been identified on lung cancer lines. Various opioid ligands, including enkephalin, were found to inhibit lung cancer growth in vitro. The opioid peptides are believed to be part of an endogenous tumor suppressor system (20).

B-16-BL6 Melanonia

Both methionine enkephalin as well as leucine enkephalin diminished the growth and number of spontaneous pulmonary metastases in C57BL6J mice implanted with B16-BL6 melanoma. At the same time, it was demonstrated that the enkephalins increase the natural killer cell activity of splenic lymphocytes (21).

PYB6 Fibrosarcoma

The maximum inhibition of tumor growth achieved with methionine enkephalin was 72% on day 15 post tumor transplantation. The enkephalin was found to increase the blastogenic response to the T-cell mitogen, concanavalin A. This would suggest that an increase in cytotoxic T Cells could explain the antitumor effect (22).

L1210 Murine Leukemia

Both methionine enkephalin and leucine enkephalin were found to increase the number of survivors, compared to controls, when mice were inoculated with L1210 leukemia. In addition, the enkephalins were reported to increase blastogenesis to PHA, which would support the mechanism of increasing cytotoxic T cells that control tumor growth (23).

Neuroblastoma

Zagon and McLaughlin identified a new opioid receptor, zeta, which appears to regulate oncogenesis. The most potent ligand found to date is methionine enkephalin. Thus, tumor appearance was delayed and survival of mice was prolonged when mice were inoculated with S2OY neuroblastoma and treated with met-enkephalin (24).

Cancer Patients

Several different types of cancer patients were treated with methionine enkephalin, including lung cancer, melanoma, kaposis sarcoma, hypernephroma, and pancreatic cancer. Antitumor activity of met-enkephalin was presumably expressed as increases in cytotoxic cells (CD4, CD8, NK) as well as levels of interleukin II. In addition, increased blastogenic responses to mitogens were also recorded. Several patients reported improvements in mood (antidepressant effect) after one to two weeks of treatment with met-enkephalin (25) (1) (2) (3).

DISCUSSION

Methionine enkephalin has now been identified to be a new cytokine that is derived from T helper cells and macrophages (3). The immunological effects of met-enkephalin are similar to those seen with IL2; increases of T cell subsets, NK cells, and blastogenic responses to mitogens (26). In this regard, met-enkephalin was found to be synergistic in LAK cell production (27). Since met-enkephalin increases cytotoxic cells (CD4, CD8, and NK), it is not surprising that antiviral effects against herpes, Flu A, Japanese encephalitis, Friend leukemia virus, MAIDS, Rauscher, and AIDS were seen. Probably the most strik-

ing therapeutic effects of met-enkephalin were seen in ARC-AIDS patients with herpes, pneumonitis, and HIV. Marked reductions in symptoms of herpes, pneumonitis, and AIDS were noted. Constitutional symptoms of weight loss, fevers, night-sweats, diarrhea, thrush, and herpes, were almost completely eliminated. In addition, the patients reported improvement in mood (possible antidepressant activity). Studies of combinations of antivirals and met-enkephalin are in progress (1) (2) (3) (15).

The anti-tumor activities of met-enkephalin are also attributable to the increases of cytotoxic cells as well as activation of macrophages (25) (3). Thus, antitumor effects were seen against L1210 leukemia, B16 melanoma, fibrosarcoma, neuroblastoma, ovarian cancer, pancreatic, colon cancer, and lung cancer. Clinical studies were carried out in melanoma, lung cancer, hypernephroma, and pancreatic cancer with met-enkephalin treatment. Immunological end-points all showed significant elevation. Tumor regression was most noted in Kaposis sarcoma patients (28).

Of special interest are the studies carried out by Faith et al where they showed that NK cell activity was increased by met-enkephalin in many diverse cancers: thyroid carcinoma, acute myelocytic leukemia, small cell carcinoma of the lung, breast cancer, ovarian cancer, gastric carcinoma, and lymphoma, Hodgkins disease, and chronic myelogenous leukemia. The latter study suggests that NK cells (as well as LAK cells) may be increased in vivo in a large spectrum of patients. In addition the increases seen in cytotoxic T cells (CD4, CD8) in patients on met-enkephalin treatment gives further hope for treating solid tumors.

In conclusion, methionine enkephalin has been identified to be a new cytokine which activates cytotoxic cells that are effective against a large spectrum of viruses and tumors (2) (30). The prohormone, proenkephalin A, is found in the central nervous, neuroendocrine, and immune systems. In times of stress the prohormone is processed to met-enkephalin and other peptides. Met-enkephalin undoubtedly contributes to mind-body relationships and interactions and may explain, in part, "spontaneous healing" (32) (33).

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