OPIOIDS INDUCE APOPTOSIS: EXPERIMENTAL EVIDENCE AND CONSIDERATIONS

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SUMMARY

Experimental and clinical evidence has shown that opioids significantly influence the immune system. Subjects who are addicted to opioids show a decrease of their immunological defences which is certainly due to poor hygiene in their life style but is also caused by other co-factors, such as a direct action of the opioids. In vitro studies have shown that opioids influence the innate as well as the specific immune function. In particular, they induce a decrease in the production of the antibodies. The apoptotic action of the opioids, which was demonstrated on the neural substrate, has also been shown in bone marrow derived cells such as macrophages, fibroblasts, and above all lymphocytes. These cells have opioid receptors on the surface. The present study evaluated the apopotic effect of mu receptor agonists (morphine and buprenorphine) and k receptor agonists (pentazocin) on the leukemic T cells. The effect was antagonized (could use a better word here ?) by naloxone to confirm a receptoral mechanism. The apoptotic effect was evaluated by detecting the hypodiploid peak (showed through iodide propitio) by means of a cytofluorimeter. The results showed that both morphine and buprenorphine induced a significant hypodiploid peak which was antagonized by naloxone. Also, pentazocin induces an analogous effect (again antagonized by naloxone) even if the peak was lower. In conclusion, one of the principal ways through which opioids influence the immunological system could be the induction of the immune cell apoptosis and this phenomena seems related to direct receptoral mechanism.

Key Words: Apoptosis, Opioids, Immune System

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INTRODUCTION

The subjects who have a severe addiction of opioids, often show a great sensitivity to infections. Intravenous injection, used in the past to administer the narcotics, were usually performed in unsanitary conditions. This behaviour predisposes the transmission of the infectious agents, which are the etiological factors of severe diseases which frequently occurred in these patients. Other causes, however, have been suggested as possible co-factors in the pathogenesis of the infection disease in drug addicts. An important pathogenetic aspect could be an early decrease of the immunological defence which is often present independently of the occurrence of the clinical signs of AIDS. The reduction of the immunological defence could be a co-factor which may facilitate the progression of the disease. The recognition of the potential co-factors become crucial.

In this study, the direct effect of the opiods on the activity of the immune system has been considered, showing their role in the susceptibility of the drug addicts to the infection diseases.

The confirmation and nature of the immuno-modulation of the opiods has been debated for years. Recent studies have shown that the opioid receptors on the immune cell have an important role in the regulation of the system (1). The opioids appear to modulate both the innate (inflammatory reaction) as well as the specific immunity. Evidence shows that some phases of the phlogosis, such as the chemotaxis and the phagocytosis of the neutrophil granulocyte and macrophages are attenuated by the opiods that exert a weak anti-inflammatory action (2). There are important indications for the effects of the opioids on the specific immunity. The opiods have an inhibitory action on antibody production (umoral immunity); they have also an effect on the cell- mediated immunity (3,4) One of the principal mechanisms of the opiod action on the immunity system is represented by the induction of apoptosis in the lymphocytes.

The apoptosis as a regulating factor of immune system.

Apoptosis, along with necrosis, is the mechanism of cell death. In apoptosis the cell death is induced by a specific programming following the activation of particular genes which induces modification of the cell structure resulting in the cell elimination. (5,6).

Apoptosis is a mechanism which could have a physiological as well as pathological meaning. The apoptosis could be induced by molecular factors which are either internal or external to the cell.

Apoptosis has a central role in the control of the various stages of lymphocyte maturation. The lymphocyte T or B, which in the tyme or bone marrow do not reach maturation through positive and negative selection are eliminated by the apoptotic mechanism.

Exogenous chemicals which induce apoptosis

Several drugs induce apoptosis, above all in tissues which have an intense proliferation capability. Drugs which are capable of inducing apoptosis are: antitumoral, steroids, cytochine, chemotherapy agents such as doxorubicine and daunorubicine.

Opiods ed Apoptosis

Clinical evidence indicates that opiod addicts are more sensitive to infection. *In vitro* studies show that morphine is capable of inhibiting, in a dose dependent pattern, the production of cytochines such as (alpha and beta interferons) induced by the HIV infection. In this model a significant increase of apoptotic features has been also reported (7,8).

The immuno-suppressive effects of the opioids has been documented. A great variety of immunological abnormality has been reported in the animal which undergoes a chronic treatment with morphine. In mice, daily injections of morhine decrease the antibody production and reduce the spleen parenchyma (9). Other studies, with chronic admistration of morphine have evidenced thymus and spleen atrophy and a decreased capability of proliferation of the T cell following phytogenetic stimuli (10,11,12).

A single dose of morphine induces a transitory modification of the immunological response (13). A single administration of heroin produces a dose- dependent decrease of leucocyte in the rat spleen. Three hours after heroin administration a significant increase of the apoptotic features is observed (14).

The presence of opiod receptors on the immune cells indicates that endorphins could be involved in the physiologic process of immunological regulation.

The mechanism which supports the apoptosis induced by opioids is not known. The evidence (that the effect is antagonized by naloxone and naltrexone) indicates that the phenomenon could be mediated by the μ receptors (15). In experimental animals, fentanyl (a synthetic agonist of the receptors) has a clear pro-apoptotic effect.

The aim of this study was to verify the apoptotic effect of opioids which are currently used in pain therapy. Also, the same effect of pentazocine was evaluated, which is a partial agonist of the k receptors.

MATERIALS AND METHODS

The study was carried out on blood samples which were drawn from three patients affected by acute lymphoblastic leukaemia (complexively 20 ml from each patient). A lymphocyte component was separated for each sample which was incubated with scalar doses of the following drugs.

Drugs

Prednison :The corticosteroids have a documented apoptotic activity and were used as comparison model. It was used a 10^{-3} dose. Morphine: scalar doses of 10^{-9}, 10^{-8}, 10^{-7} molar were used. Buprenorphine: buprenorphine is a synthetic opioid which acts on on the receptors and which is 50 times more potent than morphine. Scalar doses such as 10^{11}, 10^{-10}, 10^{-9} molar were used. Pentazocine: pentazocine is a synthetic analgesic which has a partial action on k opioid receptor. At more elevated doses it shows a morphine- like action. Scalar doses of 10^{-7}, 10^{-6},10^{-5} molar were used. Naloxone: naloxone an antagonist of the opioid receptor (both μ and k) was used 10^{-3} molar

.Experimental Methods

Each drug dose was incubed with a sample fraction (4 ml). The exposure to the drugs lasted 48 hours. The apoptotic effect was re- evaluated of the opioids after adding naloxone.

The cell cycle was evaluated by colouring with propitio iodide in hypotonic sample. The cells were detected and washed with PBS and coloured with propitio iodide which has a stechiometric binding to the DNA double strand. The percentage of the apoptotic cell is determined through the cytoflourimethric analysis of the hypodiploid nucleus. The intensity of intercalated red fluorescence in the DNA is indicative of the contained DNA.

RESULTS

The capability of prednisone of inducing a hypodiploide peak as an index of apoptosis in the lymphocyte population is confirmed in this study (Figure 1)

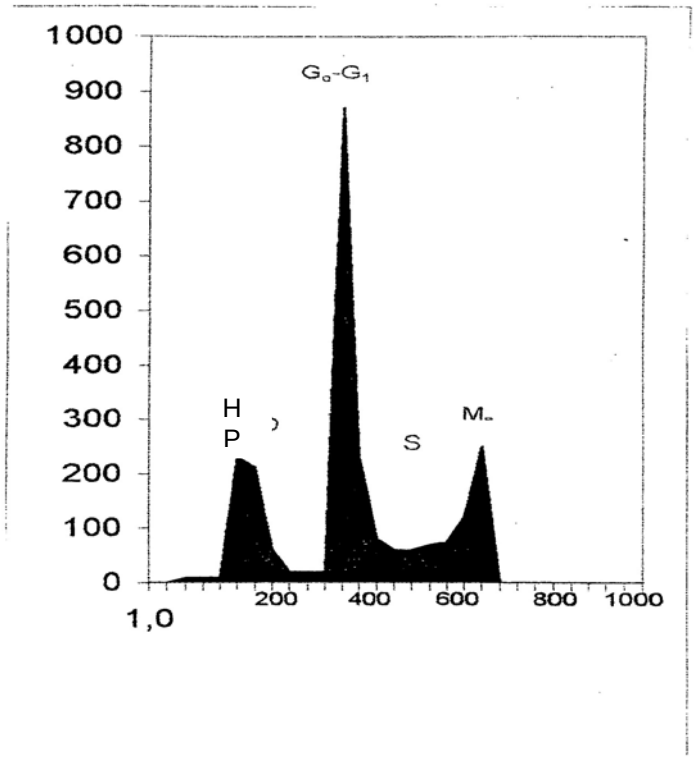

Figure 1: Predinsone ($10^{-3)}$ induced apopotsis

HP: Hypodiploide peak

Morphine was capable of inducing a significant flow of hypodiploid cell at the 10^{-7} M, even if it was lower that prednisone (Figure 2). The effect was antagonized by naloxone.

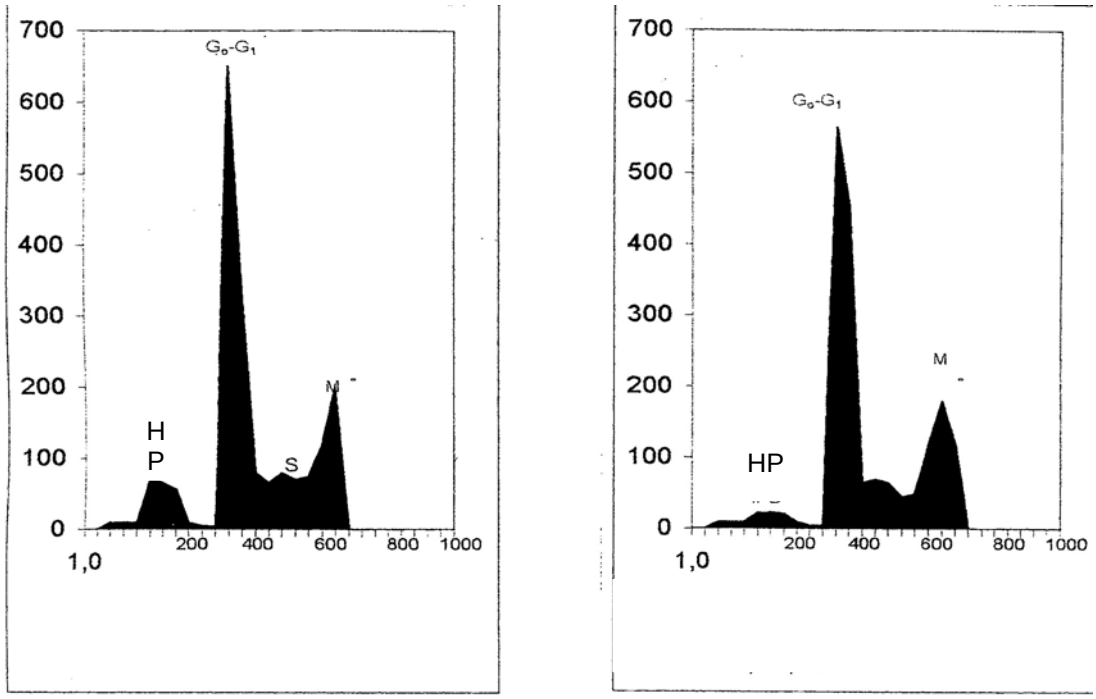

Figure 2: Morphine (10^{-7}) induced apopotsis. Effect antagnized by Naloxone

The buprenorphine induced a significant hypodiploide peak, which was also antagonized by naloxone (Figure 3).

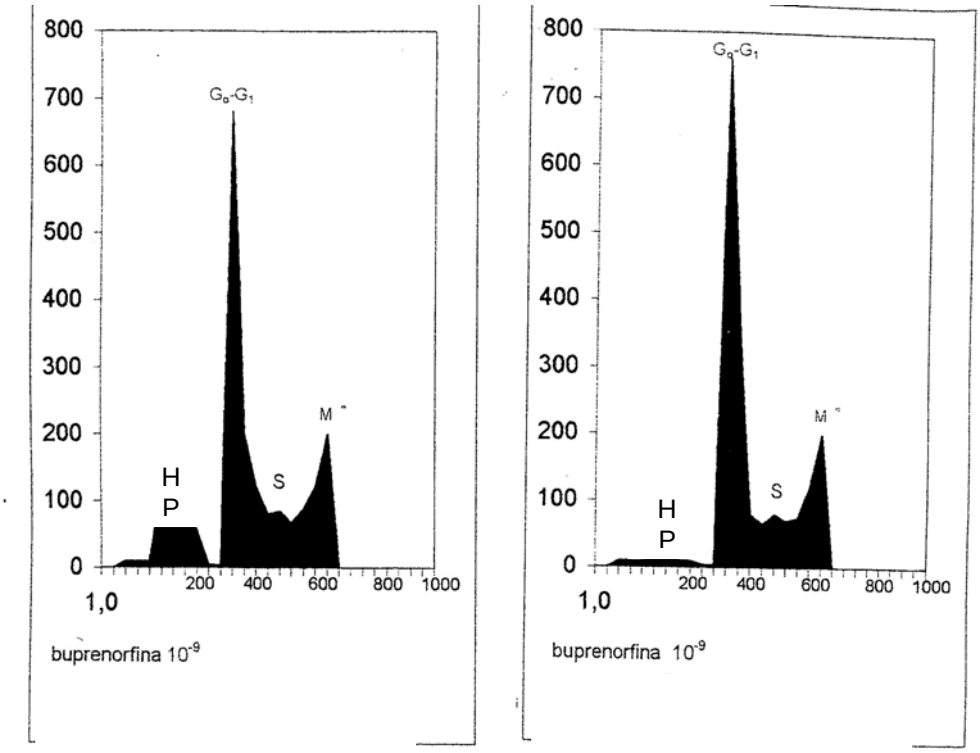

Figure 3: Buprenorphine (10^{-9}) induces apopotsis Antagonism by Naloxone

Pentazocine induced a flow of hypodiploid cells, even if less intense, indicating the presence of apoptotic phenomena (Figure 4). Naloxone antagonized also this result.

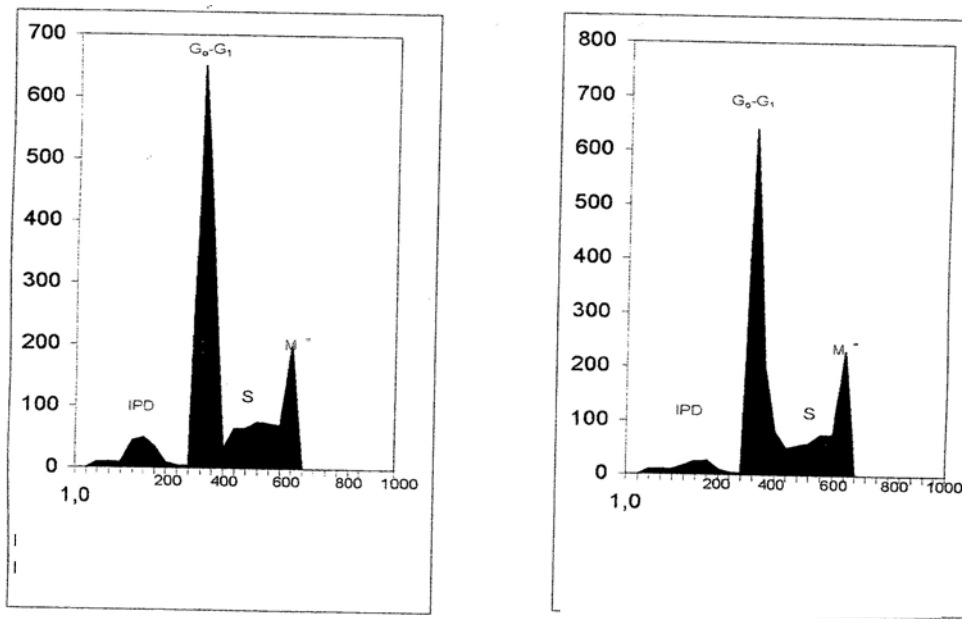

Figure 4: Pentazocine (10^{-5}) induces apopotsis Antgonism by Naloxone

DISCUSSION

This study confirms the capacity of opiods to induce apoptosis. Morphine and buprenorphine produced significant hypodiploid, emphatizing the influence of opiodergic factors on the immunitary system through the activation of the μ receptors.

An interesting result is represented by the evidence that pentazocine produces a hypodiploid peak, even if less relevant, This feature indicates that k receptors are present on the lymphocytes where they can have an apoptic effect. The control of the opioids on the immunitary system could have, then, a multireceptorial mechanism. All the apoptosis effects of the opioids are antagonized by naloxone. The evidence supports the fact that the apoptotic effect of the opioids is mediated by a receptorial activation.

The apoptosis mechanism connected to the opioids is not well determined. A recent study evidenced that the mechanism could be related to apoptotic factors which are

external to the cell such as the the binding FAS- FAS ligand (one of the principal steps in the pathways of the apoptosis). The inhibition of this binding significantly attenuates the apoptosis induced by both morphine and buprenorphine on nervous cells (16,17)

The pro- apoptotic effect related to the opioids gives rise to speculation. First, the opiods can play an important role in the regulation of the immune system, with an inhibition meaning. In this case, the endogenous opioids which are usually increased with suffering or discomfort, could contribute to the decline of the immune system observed in this situation.

The results of this study, reinforce the indication that opioids could have important role outside of the nervous system, perhaps in a multi-system network (neural, immune, endocrine) playing a role in the defense mechanism (or having a role in cell defense?)

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