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SERUM IMMUNOGLOBULINS DURING SUMMER STAY IN ANTARCTICA: ROLE OF OPIOIDS

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Abstract

The humoral immune status was investigated in six volunteers before, during and after their short stay in Antarctica during the austral summer. The role of opioids in immunomodulation and their receptor regulation was investigated by chronic naltrexone administration. The results reveal that the serum IgG and IgM concentration is unaffected during summer stay in Antarctica. The results also indicate that there might be an interaction of neuroendocrine factors with opioids or its receptors, getting activated only during the summer of Antarctica. This remains to be investigated further.

Introduction

Antarctica has the harshest living conditions, being the coldest, windiest, highest, barren and most isolated continent. An Antarctic expeditioner has to face and cope up with many physical and psychological stresses. Reports about the health aspects of persons during and after a short or long stay in Antarctica are scanty and inconclusive. Amongst the many medical problems encountered in Antarctica, of particular interest is the observed immunosuppression during Antarctic sojourn (Muchmore *et al.*, 1970; Tashpulatov, 1974; Williams *et al.,* 1986; Muller *et al.,* 1995a; Muller *et al,* 1995b).

Muller *et al.* (1995a) observed decreased cell mediated immune responses (CMI), assessed by CMI Multi-test, showing a negative correlation with anxiety in Antarctica. The association with anxiety suggests that brain peptides or associated cytokines may have a role in mediating this response.

There is a wide body of literature showing the detrimental effects of stress on the immune system (Bonneau *et al.,* 1990; Khansari *et al.,* 1990; Guillemin and Cohn 1985). On the contrary, cold stress has been shown to enhance various immunological parameters. Cold exposure enhanced the Immunoglobulin metabolism in rabbits (Sabiston and Rose, 1976) and in mice (Carr *et al.,* 1992).

Nitroblue letrazolium reduction by neutrophils was increased after sub-acute cold swim stress in rats (Sundaresan *et al.,* 1990). Yang *et al.* (1992) demonstrated enhancement of splenic lymphocyte blastogenesis in vitro of mice following their cold exposure. In Antarctica the observed immunosuppression could be primarily due to stressors like altered photoperiod or isolation in comparison to cold exposure.

It has been recognized that stress activates corticotrophin releasing factor (CRF), leading to the secretion of adreno- corticotrophin releasing hormone (ACTH) and the opioid peptides beta-lipotropin/beta-endorphin from the anterior pituitary (Morley, 1981). Since the rise in glucocorticoids is often a relatively late event during stress situations, these peptides, which are released earlier, activate immune events. Opioids have an inhibitory action on the immunoglobulin synthesis in humans (Carr *et al.,* 1990; Morgan, 1996). Animal investigations indicate that physiologic stimulation of endogenous opioid release for longer period results in inhibition of immune system (Shavit *et al* 1984; Morley *et al,* 1989; Tache *et al.,* 1989).

Life in Antarctica is stressful, which could activate the endogenous opioid release. In the present study, the role of opioids in immunomodulation during a short exposure to Antarctic conditions during the austral summer has been studied in humans.

Materials and Methods

Subjects

The study was conducted on six volunteers, who were members of the summer team of the 14th Indian Scientific Expedition to Antarctica (1994-95). Their mean age was 35.5 + 5.32 years and mean weight 67.25 ± 9.9 kgs. In these subjects morning blood sampling was done to assess the serum IgG, IgM and Cortisol. The pain threshold time was assessed using the cold pressor test. These parameters were studied before and after opioid blockage using Naltrexone (NLT).

All the subjects in the study were volunteers only. The ethical considerations set by the Ethical Committee of All India Institute of Medical Sciences were strictly followed. The subjects were given the freedom to drop out at any point of the study, if they felt so.

Naltrexone (NLT) Administration

50 mg of Naltrexone (Trexon, DuPont) tablets were given orally for eight days to effect opioid blockage. Administration was done in the evening hours.

Blood collection and pain threshold time were measured on the morning of the ninth day. Naltrexone is reported to be a pure opioid antagonist, possessing no opioid agonist properties (Ginzburg & MacDonald, 1987).

Oral administration of Naltrexone results in rapid absorption with peak plasma concentrations of 19 to 44 μg/L being reached within 1 hour. The mean elimination half-life have been reported to range from 1.1 to 10.3 hours for orally administered naltrexone (Gonzalez and Brogden, 1988). The antagonist doses chosen were previously shown to block completely many opioid mediated effects in different organs and parts of the body (Manfredi *et al.,* 1993).

Experimental Phases

The parameters were recorded in three phases. First on board ship while in the warmer waters, which forms the basal (23° 7' S, 56°5' E, LTD 13:11, 24 ± 2 °C). Second, at summer camp of Maitri, after three weeks of Antarctica stay during the austral summer (70°5' S, 11°4' E, L/D 23:01, -10.5 °C). Third, on board ship during the return voyage after the ship left Antarctic cold waters and moved into warmer latitudes (26°5' S, 54°6' E, L/D 13:11, 26 ± 3 °C).

Procedures

Enzyme Linked Immunosorbant Assay (ELISA) of Immunoglobulins

The method of Kaur *et al.,* (1991) was adopted. The standard Immunoglobulin G or Immunoglobulin M (DAKO) or the test sera optimally diluted in 0.5 M Carbonate Bicarbonate Buffer (pH 9.6) were coated in triplicate wells on to the solid phase (COSTAR ELISA plates, 96 well, flat bottom) and incubated at 37 °C for 4 hours in a humid chamber. After decanting and washing with Phosphate Buffered Saline (PBS 0.1 M; pH 7.25), non specific binding was blocked using skimmed milk powder (Anikspray) by incubating at 37 °C for one hour. Following PBS-Tween washing And Human Ig-HRP conjugate (DAKO) was then added and incubated for one hour at 37 °C. The chromogen (Ortho Phenylene diamine dihydrochloride [OPD], Sigma) dissolved in the Substrate buffer (1.5 M citrate phosphate buffer; pH 5.0) was used to develop the color and the reaction was stopped using 8N H_2SO_4; The optical density (OD) was then read in an ELISA reader (Pharmacia), at 490 nm.

Radio Immuno Assay (RIA) of Cortisol

The IMMUNOTECH CORTISOL assay kit (Immunotech, France) was used for the quantitative determination of Cortisol. This method employs highly specific monoclonal antibody coated tubes. Test serum, the control and stand-

ards were incubated in monoclonal antibody-coated tubes with ^{125}I-labeled Cortisol tracer. After Incubation, the liquid contents of the tubes were decanted and bound radioactivity was measured. Unknown values were calculated from the logit-log transformation of the counts.

Pain Threshold Time — By Cold Pressor T est

The latency of response to a noxious stimulus, cold in this study, was measured and interpreted as an index of pain tolerance (Dowling *et al.,* 1983). The subjects were asked to immerse their non-dominant hand in cold water (6 ± 0.5 °C) upto the level of wrist with the palm kept open. They were told to take out the hand at the point at which they cannot bear the pain. The length of time (in seconds), the subject will bear the pain was noted as the Pain threshold time.

Statistical Analysis

The results are expressed as the average values with standard deviation (mean ± SD). Statistical significance was determined using ANOVA and paired 't' test.

Results

Serum IgG

The serum IgG remained without any significant changes in the three phases studied except for an insignificant decrease in the concentration during the return voyage (from 1487.63 ± 148.44, basal to 1068.62 ± 150.53, return) after about 2 months of stay in Antarctica during the summer period. Following naltrexone administration the serum IgG concentration decreased significantly ($p<0.05$) during the summer period in Antarctica and such a decrease was not seen in any other phase (Table-1).

Table 1: Immunoglobulin G (mg/dL)

Treatment	Basal	Summer	Return
Before NLT	1487.63 ±148.44	1505.43 ±109.92	1068.62 ±150.53
After NLT	1507.61 ±171.38	1277.39 ± 186.99 bI	1160.50+116.11

a = In comparison with basal; b= Before Vs After NLT; l=$p<0.05$,2=$p<0.01$ 3=$p<0.001$

Serum IgM

The serum IgM levels were unaltered either by Antarctic exposure or by opioid blockage **(Table-2).** There were no changes in these values even after opioid blockage.

Table 2; Immunoglobulin M (mg/dL)

Treatment	Basal	Summer	Return
Before **NLT**	111.18 ± 15.30	**104.40 ± 7.59**	**103.65 ±11.67**
After NLT	**113.07 ±19.05**	**114.18 ±14.64**	**108.56 ±12.62**

Serum Cortisol

The Cortisol values were comparable to the basal values while at Antarctica and during the return voyage. Following opioid blockage there were no significant Changes. (Table-3).

Table 3: Cortisol (nMoI/L)

Treatment	Basal	Summer	Return
Before NLT	276.94 ± 65.35	302.14 ± 128.08-	303.07 ±128.38
After NLT	249.19± 110.62	167.36 ±147.30	231.17±50.07

Pain Threshold Time

The pain threshold time increased significantly from the basal values of 39.33 ± 6.62 to 128.78 ± 50.37 sec. while at Antarctica (p) which decreased to 69.45 ±51.29 sec. during the return voyage. During the summer of Antarctica, chronic Naltrexone administration decreased the pain threshold time slightly from 128.78± 50.37 to 99.55± 59.58 sec. but this decrease was statistically not significant (Table- 4).

Table 4: Pain Threshold Time (Seconds)

Treatment	Basal	Summer	Return
Before NLT	39.33 ±6.62	128.78 ± 50.37 a2	69.45 ±51.29
After NLT	47.44 ± 20.23	99.55 ±59.58	70.54 ±51.45

a = In comparison with basal; b= Before Vs. After NLT; l=$p<0.05$, 2=$p<0.01$ 3=$p<0.001$

Discussion

The present study reveals that the serum concentrations of IgG and IgM are not inhibited during or after a short stay in Antarctica during summer. No direct role for the endogenous opioids in modifying serum immunoglobulin

concentration is indicated even in a stressful environment like Antarctica where higher level of endogenous opioid concentration is expected.

Chronic naltrexone administration has been shown to cause up-regulation of opioid receptors in mice (Yoburn *et al.,* 1989) and in splenocytes of humans (Manfredi *et al,* 1993) under normal and non-stressed conditions. In the present study it was tested whether the up-regulation can occur even in a stressful situation such as Antarctica where the endogenous opioid levels are expected to be higher. To find out whether there is up-regulation of opioid receptors following chronic naltrexone administration, indirect evidence was derived from the serum Cortisol levels. It is well known that the opioid peptides have a tonic inhibitory tone on the release of corticotrophin releasing factor through noradrenergic brainstem pathway (Grossman, 1989). In normal conditions opioid blockage results in increased serum Cortisol levels (Inder *et al.,* 1995; Delitala *et al,* 1994; Grossman, 1989).

The results indicate that the up-regulation of opioid receptors occurred in all the three phases studied, but this affected the serum immunoglobulin concentration only during the Antarctic summer along with a mild decrease in the Cortisol and pain threshold time. These observations indicate that there might be one or more factors acting through the opioid receptors getting activated during the summer of Antarctica. The nature of this factor remains to be investigated.

Exposure to various behavioral or physiologic stressors can delay or block responses to painful stimuli. Activation of endogenous opioid systems by real or perceived stressors may provide endogenous pain relief (Holaday *et al.,* 1989). However our results of pain threshold obtained before and after naltrexone administration indicate only a partial role for the opioids in pain tolerance to cold while at Antarctica. The increased cutaneous vasodilator responses in chronic cold adaptation (Naidu and Sachdeva, 1993) may also serve as an explanation for the observed significantly higher pain threshold time during summer stay.

In conclusion, our results show that the humoral immune response is unaffected during the summer stay in Antarctica and the endogenous opioids do not seem to be having a direct role in modifying the serum immunoglobulin concentrations during Antarctic summer.

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