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Research Article

Decreased Brain Serotonin Level in Adjuvant Induced Arthritic Rats

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ABSTRACT
Back ground: Rheumatoid arthritis is an autoimmune disorder in which patient not only suffered from joint misery but also face its associated depression. *Objectives*: To determine brain serotonin levels in adjuvant-induced arthritic (AIA) rats as its decrease concentration contribute to make body depressed. *Methodology*: AIA was induced in female Sprague-Dawley rats. Paw volumes was measured to evaluate arthritic progression while brain serotonin was estimated by HPLC-EC method. Data was analyzed by student t-test. *Results*: Significant reduction ($p<0.005$) in all brain serotonin level was found in arthritic rats when compared with normal. *Conclusion*: Brain serotonin level decreases in AIA rats which may be one of the reasons of its depression.

Key words: Adjuvant induced arthritic rats, serotonin, HPLC

INTRODUCTION

Among chronic pains, rheumatoid arthritis (RA) holds the predominant position [**1**]. It is a chronic symmetrical poly arthritic progressive inflammatory disorder [**2**] in which patient has to face long-term pain, stiffness and fatigue due to hyper activity of immune system mainly T and B-lymphocytes [**3-4**].

In addition to this, RA patient also suffered from depression and its frequency is about twice than healthy one [**5**]. Alliance of depression in this group is unified with agitated activity of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) [**6-8**] which affects a number of mechanism in brain as reduce brain monoamines activity [**6**], activation of stress-induced or mitogen-induced protein kinases, Janus kinase or signal transducers and activators of transcription[**5, 9-12**]. It was reported earlier that level of tryptophan, which is the precursor of serotonin, in RA patient decreases due to its increase catabolism by indoleamine (2, 3)-dioxygenase enzyme released from interferon-γ in inflammatory cell. Therefore its availability in brain decreases which may ultimately decreases serotonin synthesis in brain. Since serotonin is the neurotransmitter which is involved in mood, consciousness and sleep therefore decrease concentration of serotonin makes the arthritic body depressed**.** [**9-14**]. Therefore determination of this biologic amines in RA patients and animal models of RA help to understand neurological disorder as anxiety and depression related to this pathology [**15**].

The principle aim of this work was to study the involvement of serotonin on the depression associated with RA. For this purpose adjuvant induced arthritic (AIA) rats were used as animal model that having similar pathological features as RA in human [**16-17**].

MATERIAL AND METHODS

Animals: Female Sprague–Dawley rats, weighing 215–230 g (8–10 weeks), kept at 21 ± 2°C on a 12-hour light/dark cycle with free access to standard laboratory rat food pellets and water, were used for this study under the ethical guidelines of International Association for the Study of Pain in conscious animals. Rats were randomized and grouped (n=6) into healthy (normal) and adjuvant induced arthritic rats (AIA).

Induction of arthritis: For this study, chronic model of arthritis i.e. arthritis induced by adjuvant was selected. Arthritis was induced by intradermal injection of 0.1ml suspension of 1mg of fresh Lyophilized *Mycobacterium tuberculosis* H37Ra (MT H37Ra; DIFCO Laboratories, Detroit, MI, USA) in liquid paraffin oil into the tail base of all the rats of AIA group, using a sterile hypodermic needle [**1**]. This day was considered as day zero and observation was continued for 3 weeks until arthritis was fully developed in AIA group. Clinical assessment on progression of adjuvant induce arthritis:Measurement of rat's hind paw volume was used to evaluate arthritic severity produced by the adjuvant administration. It was determined by quantitating the change in their paw volume on alternate days throughout the experiment by water dislocation procedure with the help of plethysmometer (model 7140; Ugo Basile, Varese, Italy) which has the capability to measure paw tibiotarsal joint three in dimensions. Thus any variability of the pattern of swelling of individual limbs can be monitored.

Brain dissection technique: Rat's brain samples were

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Table 1: Paw edema in AIA rats

Days	Normal	AIA control
0	4.19±0.18	4.18±0.38
2	4.19±0.10	4.195±0.25
4	4.2±0.14	4.195±0.31
6	4.21±0.65	4.21±0.68
8	4.21±0.39	4.21±0.37
10	4.22±0.015	4.225±0.42
12	4.22±0.17	*4.30±0.47
14	4.22±0.11	*4.305±0.78
16	4.23±0.11	*4.444±0.56
18	4.24±0.18	**4.74±1.8
20	4.243±0.82	**5.74±4.2
22	4.51±0.63	**5.89±4.6
24	4.51±0.65	**6.1±3.2
26	4.51±1.02	**6.275±3.3

Values are mean ±S.D.Data analyzed by student t-test.* and ** indicate significant ($p<0.05$) and highly significant ($p<0.005$) form normal.

collected to estimate brain serotonin levels. For this purpose, on final day of experiment rats were chopped and brain were quickly excised (within one minute) from cranial cavity removing duramater. The brain extending upto frontal cortex rostrally and medulla oblongata caudally was dipped in chilled saline and stored at -80^0C until assay of serotonin was conducted. Extraction of indoleamines from brain: The brain serotonin from the frozen rat's brain was extracted by electric homogenizer using 0.4M perchlorate as an extraction medium. Homogenates were then allowed to stand for 10-15 minutes for the precipitation of proteins. Supernatant was decanted in a separate eppendrof tube and centrifuged at 12,000 rpm for five minutes. After centrifugation supernatant was separated and used for the estimation of serotonin (5-HT).

Table 2:*Brain Serotonin levels in AIA rats*

Groups	Serotonin(ng. gm^{-1})
Normal	1246.8±37.5
AIA	974.8±18.9

Values are means± S.D taking $p<0.005$ as highly significant figure from normal by student t-test

Estimation of brain Serotonin: Concentration of serotonin is very low in brain sample that can typically estimated by RP-HPLC method coupled with electrochemical detector. Its level was estimated as previously described [18] to study the influence of serotonin on RA associated depression. Briefly, a 5II Shim-Pack ODS separation column (4.0 mm * 150 mm) was used and separation was accomplished by a mobile phase containing methanol (14%), Octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer at pH 2.9 at an opening pressure of 2000-3000psi on Schimadzun LEC 6A detector at an operating potential of 0.8V for serotonin.

Statistical analysis: Data was analyzed by student t-test using SPSS INC. software taking significant level $p<0.05$.

RESULTS

Arthritic determination: During experiment evidence of clinical tenderness was instigated to observe from day 10, showing erythmia in joints especially in ankle, metatarsal and interphalangeal joints in AIA group which became significant (p=0.032) from day 12 in contrast to normal. Table 1.

Effect on brain serotonin :In the AIA that is arthritic groups, brain serotonin levels decrease significantly ($p<0.005$) when judge against with normal (table 2).

DISCUSSION

Previously studied showed that RA patient also suffered from depression and anxiety along with joint disorder [**5**]. Among many causes of depression [**5, 9-14**] previous research indicated that depletion of brain serotonin also play important role in severe depression and anxiety [**19-20**]. Therefore the principle aim of that present work was to study the involvement of serotonin in RA associated depression. For this purpose experiment was performed on adjuvant induce arthritic (AIA) rats which represents chronic model of inflammation. Results showed same consistency that use of fresh Lyophilized *Mycobacterium tuberculosis* H37Ra (MT H37Ra; DIFCO Laboratories, Detroit, MI, USA) as adjuvant produce severe arthritis as indicated in AIA group as indicated in table 1 [**1**]. Clinical tenderness was activated from day 10, showing erythmia in joints especially in ankle, metatarsal and interphalangeal joints in AIA group which became significant (p=0.032) from day 12 and fully developed as all the rats in that group showed severe fore and hind paw erythmia in contrast to normal.

Previous studies also showed the existence of neurological disorders as depression and anxiety in RA patient which is also associated with hyperactivity of pro-

inflammatory cytokines during inflammation [**6**]. It was also reported that tryptophan is an essential amino acid and being the precursor of serotonin is involved in mood, consciousness and sleep, it is mainly metabolized in liver by kyneurenine (Kyn) pathway through tryptophan pyrolase enzyme while remaining in brain [**21**]. In addition to normal metabolism of tryptophan in RA patient, it has also been reported that levels of plasma tryptophan further decreases due to its increased additional catabolism by indoleamine (2, 3)-dioxygenase enzyme released from interferon-γ in inflammatory cell during arthritis. This extra increase in tryptophan catabolism in RA patient may effect tryptophan transportation into brain via common carrier system located on blood brain barrier [**22**]. In the present work, brain serotonin level decreased significantly ($p<0.005$) in the entire AIA arthritic groups when judged against normal healthy rats. Therefore the possible explanation of low brain serotonin level is the decrease tryptophan availability in the brain which may contribute to the decreased serotonin synthesis at this region [**23**]. Our results indicated that decreased serotonin level in brain might be one of the important reasons to make the arthritic body depressed [**19**].

CONCLUSION

We want to highlight here that among many reason for the depression associated with RA, decreased brain serotonin level may be one of major reason of this RA related pathology.

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