MOLECULAR DOCKING ANALYSIS ON THE IDENTIFICATION THE ACTIVATION OF ENDOGENOUS BETA ENDORPHIN ON THE REGULATION OF NEURO INFLAMMATORY SIGNALLING RELEVANT TO INFLAMMATORY MEDIATED TUMOR

Running Title :- Interaction of beta endorphin with the neuro inflammatory signalling

Type of article :- Original Research

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ABSTRACT:

BACKGROUND: Beta-endorphin is an opioid neuropeptide which has an important role in paracrine communication of brain messages. Beta-endorphin is the link that is missed between the neuron and the wall of the arteriole, it is a fundamental neurotransmitter. When you exercise, a chemical is released by the body called beta-endorphins which interact with the receptors in your brain to reduce the perception of pain.

AIM: To analyse the beta endorphin interaction with inflammatory markers and also to identify the activation of endogenous beta-endorphin on the regulation of neuro inflammatory signalling.

MATERIALS AND METHODS: The molecular docking analysis is a bioinformatic study conducted in a private dental college. The endogenous substance Beta-endorphin which is secreted after exercise is used as a target protein.The interaction of beta-endorphin with the proteins relevant to inflammatory mediated tumor namely IL-6,TNF-alpha, MMP are included for docking analysis. The protein structure is retrieved using protein data bank, protein protein docking done using patch dock server followed by visualization of protein-protein interaction using pymol.

RESULTS: The representation of beta endorphin with IL-6, TNF-alpha, MMP complex showed good shape complementarity. The amino acids THR-16, LEU-17, PHE-18, LYS-19, ASN-20, ALA-21, ILE-22, LYS-24, ALA-26, TYR-27, GLY-30, AND GLU-31 of Beta endorphin were found to be involved in the interaction with IL-6, TNF-alpha, and MMP.These residues are also present in the active site region of beta-endorphin.

CONCLUSION: From the results of the analysis it can be concluded that the role of exercise induced beta endorphin may act as a regulator of IL-6, TNF- alpha signalling in inflammatory mediated tumor.

KEY WORDS:
Tumor; beta-endorphin;TNF-alpha; IL-6; MMP; inflammatory mediated tumor;innovative method.

INTRODUCTION:

There are two types of inflammation related to tumor 1) pretumorous inflammation lesions 2) inflammation present in all tumor tissues. There are two pathways: extrinsic mechanism, intrinsic mechanism. Inflammation is always associated with tumor. Intrinsic means tumor elicited inflammation through tumor initiating mutations. Extrinsic means bacterial and viral infections, tobacco smoking(1). Exercise induced modulation of the immune system which tumour initiation and progression. Exercise modulates the number of cell innate and adaptive immunity(2).

Chronic inflammation is malignant cellulase transformation. Exercise and inflammation in tumor favorably alter numerous components of the immune system, modulating tumorigenesis(3) (4). Inflammation and development progression of tumor increase body's response and tissue damage mechanism and mutagenic potential of inflammation tumor cachexia causes muscle wasting and mitochondrial dysfunction and mechanism associated with tumor(5). The experience from our previous studies (6) (7,8) (7)(9)(10)(11)(12)(10,12)(13)(14) (15) have led us to concentrate on the study.

The anterior pituitary gland stores a large amount of beta-endorphins. Inflammatory mediators such as IL-6 and TNF are induced by neurohormones. -alpha activate key transcription factors which promote angiogenesis, immunosuppressant and metastasis leads to tumor(16). Endorphins are endogenous morphine that are responsible for the "runners high," a relaxed psychological state. Inflammatory cytokines were reduced in the inflammatory tumour microenvironment in mice treated with beta endorphin cells., thus inhibiting tumour growth. Exercise can be one of the most important tumor treatments(17). Studies at molecular levels were performed by our team of researches which insisted us to proceed this study (18–25),(26),(27),(28),(29,30),(31),(32),(33–37) Aim is to assess the beta endorphin interaction with inflammatory markers and also to identify the activation of endogenous beta-endorphin on the regulation of neuro inflammatory signalling.

MATERIALS AND METHODS:

Retrieval of Target proteins structures from Protein data bank

In order to study the mechanism of interaction between Beta endorphin with IL-6, TNF-alpha, MMP proteins, the three dimensional structures were downloaded from Protein Data Bank using the respective ids (Pdb ids: Beta endorphin- 6TUP, IL-6- 1ALU;TNF-alpha -2AZ5; MMP-4AU0) (38)

Protein-Protein Docking

The interaction of Beta endorphin with IL-6, TNF-alpha, and MMP proteins was studied using a geometry-based molecular docking technique called Patch Dock (http://bioinfo3d.cs.tau.ac.il/PatchDock) [2,3]. The Patch Dock service generates docked transformations with high molecular shape compatibility. The programme divides the Connolly dot surface representation of the molecules into concave, convex, and flat patches. In order to produce various transformations, the patches were connected according to their complementarity. For clustering, a default value of 4 Å was used and redundant solutions were discarded by RMSD clustering. The geometric score, desolvation energy, interface area scale, and the actual rigid transformation of the solutions are created by the Patch Dock output. For each complex, twenty solutions were created, from which one complex was selected for further analysis based on the scoring geometric shape for both complexes.

Visualization of Protein – Protein interactions.

Using the academic version of the Pymol, the residual interactions between docked complexes were viewed. The intensity of colour for interactions was readily visualised and exported for findings [4]. Pdbsum was used to find out what kinds of interactions Beta endorphins have with IL-6, TNF-alpha, and MMP proteins.

RESULTS:

The three dimensional structure of these proteins were separately uploaded in the patch server. The top 20 docked complexes were listed once the docking was completed. Among them based on the scoring parameters best one docked complexes of each protein were selected for further analysis. This docked complex was visualized by using pymol software. The amino acids THR-16, LEU-17, PHE-18, LYS-19, ASN-20, ALA-21, ILE-22, LYS-24, ALA-26, TYR-27, GLY-30, AND GLU-31 of Beta endorphin were found to be involved in the interaction with IL-6, TNF-alpha, and MMP. These residues are also present

in the active site region of beta-endorphin. So it was confirmed that these proteins IL-6, TNF-alpha and MMP might activate the Beta-endorphin and play a main role in the regulation of neuro-inflammatory signaling.(Table 1)

TABLE 1: Molecular docking results of beta endorphin with IL-6, TNF-alpha and MMP proteins.

PROTEIN NAME	SCORE	ACE (atomic contact energy)
IL-6	12682	-397.34
TNF-alpha	16966	-424.00
MMP	16108	-332.40

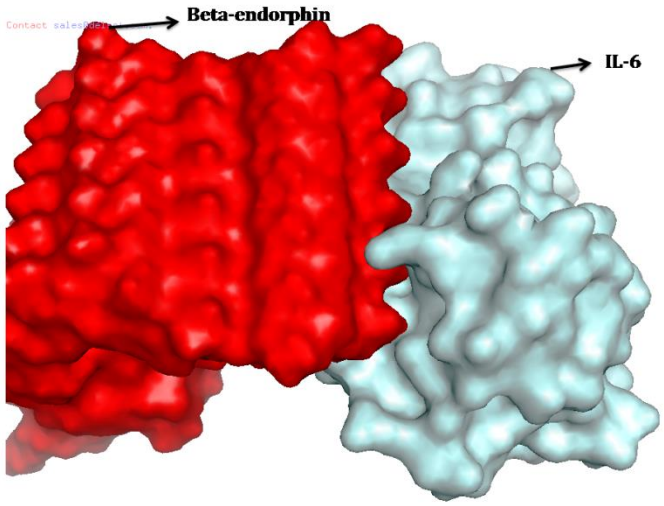

Figure 1: The figure represents the protein -protein interaction between the IL-6 and Beta endorphin.

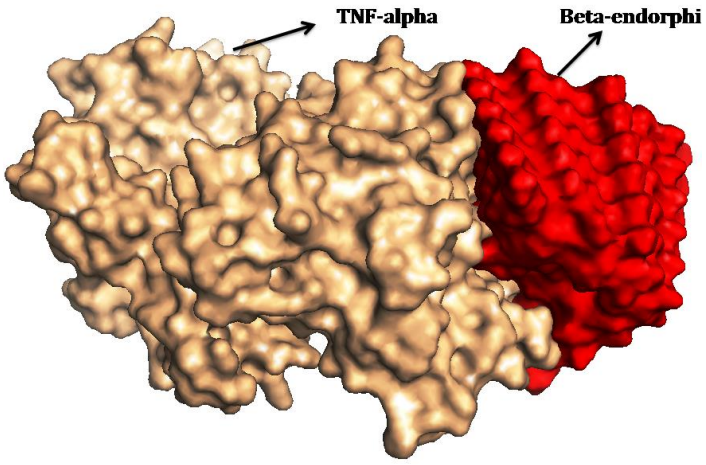

Figure 2: The figure represents the protein protein interaction between TNF-alpha and Betaendorphin.

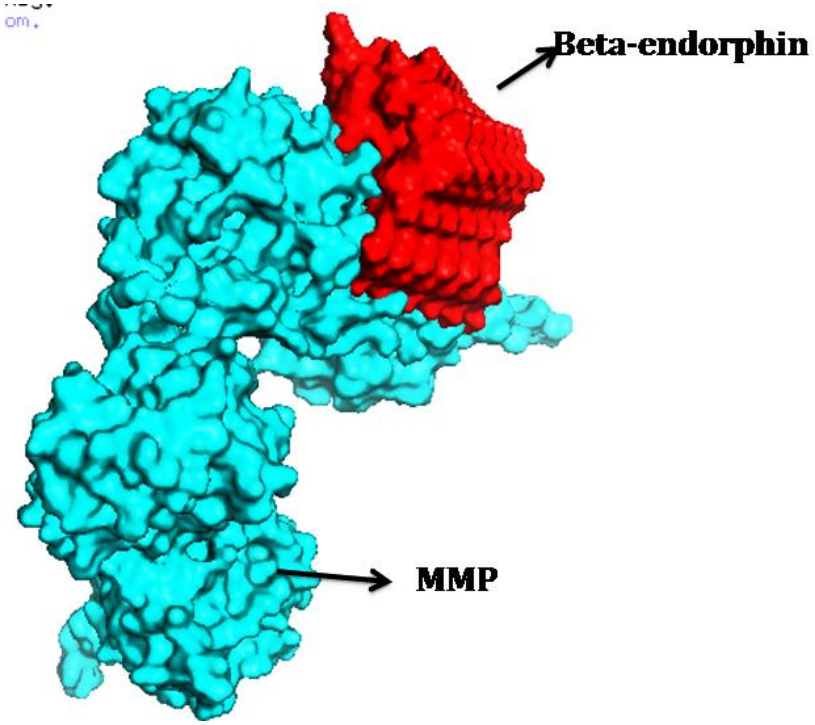

Figure 3: The figure represents the protein protein interaction between MMP and beta-endorphin.

DISCUSSION:

The present study is evidence that exercise-induced endogenous beta endorphin may act as a protective compound against inflammatory mediated tumor.There is a positive interaction between the beta endorphin and IL-6, TNF-alpha and MMP. Immune cells secrete various interleukins which regulate the cell growth and motility. The important role is stimulating immune responses, such as inflammation(39). Tumour necrosis factor is an extraordinary pleiotropic cytokine with a role in immune homeostasis and inflammation. TNF-alpha causes inflammation by triggering the production of immune system molecules like IL-1 and IL-6.Tumours utilize MMPs to cleave chemokines by preventing inflammatory cell chemotaxis.

The basic building blocks of life formed by amino acids are proteins. The amino acids are gene-coded and form peptides, P peptides are further formed proteins, and living tissues are formed by proteins. In addition, proteins also play a key role in biological processes, such as catalytic reactions, molecular transmission, immune reactions to different pathogens, and cell-to-cell signal transduction. The biological activities described above are regulated by protein complexes, which are usually mediated by protein-protein interactions (PPIs). "In cells, PPIs form a dynamic network with a concept called "interactome". In physiological and pathological processes, the interactome has an important function, including signal transduction, cell proliferation, development, differentiation, and apoptosis, etc. Therefore, many human illnesses, such as tumor, infectious disorders and neurodegenerative diseases, are linked with aberrant PPIs. Since enzymes, ion channels, or receptors are typically the classic drug targets, PPIs suggest new possible therapeutic targets. PPIs have gained growing recognition in recent years and have become appealing targets. Recent studies suggest that PPIs have tremendous potential as an intervention target for

new refractory disease therapy, and their control is generally regarded as a promising drug development technique. Identification of Protein –protein complex in experimental methods is time consuming and cost effective. Nowadays so many computational methods are available to identify the interaction between the two targets for proteins Patch dock is one of the most used docking servers for protein-protein docking. It's a molecular docking algorithm based on geometry. Its goal is to find molecular shape complementarity-friendly docking changes. When such transformations are used, they produce large interface areas as well as minor quantities of steric conflicts. The interaction of Beta endorphin with IL-6, TNF-alpha, and MMP proteins was investigated in this study. dock repair.

The most prevalent endorphin, beta endorphin, is more powerful than morphine. POMC is generated and secreted in the anterior pituitary gland, and it acts as a precursor to it. Analgesic and anti-inflammatory mechanisms, for example. With despair, fear, and hatred releases of CRH from the hypothalamus, chronic psychological stress is a predisposing factor for tumor. Through the SNS activity of ANS release neurohormones like cortisol, ACTH, and noradrenaline(40,41). These neurohormones activate mediators such as IL-6, which in turn activates the STAT-3 transcription factor, which promotes tumour development. (40).

Biochemical and immunological characteristics measured by immuno enzymes. Study revealed inverse correlation between beta endorphin levels and those of leptin, TNF-alpha.. Anti-inflammatory cytokines such as (IL-8, IL-12) were increased in beta endorphin cell-treated rats, while inflammatory cytokines were suppressed (TNF -alpha) (42)(43).

Tissue damage is caused by proteolytic enzymes such as MMP-2,9, and all of these alterations lead to tumour development, invasion, and metastasis. (44) . Later results in tissue damage and cellulase changes by activating matrix metalloproteinases leads to autoimmune They are required for epidermal differentiation and the prevention of wound scars, and their overexpression promotes ageing and tumor. (45). MMP are produced by many cells including lymphocytes and granulocytes.As it is a computer based study, feature researches should be done in invitro and in vivo

CONCLUSION:

From the results of the analysis it can be concluded that the role of exercise induced endogenous beta endorphin may behave as a regulator of IL-6, TNF- alpha signalling in inflammatory mediated tumor.

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CONFLICT OF INTEREST:

All the authors declare that there was no conflict of interest in the present study.

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