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Full Length Research Paper

Clinical/computational investigation on post traumatic heterotopic ossification in head injury patients; the positive and negative role of temperature in physical treatment

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Bone morphogenetic proteins (BMPs) induce bone formation and healing. Bone can form in extraskeletal tissue in response to trauma and elevating BMPs. Several BMPs stimulated to increase due to neuroprotective effect. To get the injured zone of fraction warm is one of the suggested methods to prevent heterotopic ossification (HO). But about half of patients get the worst clinical situation. Montecarlo simulation was done with Hyperchem 8 and Ramachandran plot were designed with VMD 1.8.2. pdb files of both 1 tfg and 2 tgi proteins were selected from Protein Data Bank. φ and ψ of all 110 amino acid were calculated in both proteins after Montecarlo simulation at 290, 310 and 315 K for 200 ps, surrounded by water. 1 tfg is affected by increasing the temperature. The favored and allowed regions were decreased in population of dihedral angles. Increasing the temperature more than room temperature can cause more allowed dihedrals for 2 tgi. It is suggested that utilization of thermogenerator lamp progress the HO in such patients.

Key words: Transforming growth factor, bone morphogenic proteins, post traumatic heterotopic ossification.

INTRODUCTION

Bone formation and healing study is considered as a multidisciplinary field that applies principles of Biochemistry, Cell biology, Histology and Orthopedics surgery sciences, towards the development of biological substitutes for the restoration, maintenance or improvement of tissue form and function (Sanders et al., 2010; Ruimerman, 2005). Bone is a living tissue and its contains proteins, calcium phosphate and is capable of regeneration and remodeling. Bone formation occurs in a dynamic integration of biochemical, cellular and hormonal processes that are facilitated by states of bone deposition, bone resorption and bone remodeling (Ruimerman, 2005). Specific bone-inductive proteins can, induce bone formation and healing in vivo. Bone can form in extraskeletal tissue in response to trauma (Sanders et al., 2010; Atkinson et al., 2010). This initiated research into bone regeneration by putative soluble signals, led to the discovery and identification of the effect of temperature on the 1tfg (Figure 1a) and 2 tgi (Figure 1b) growth factors entirely novel family of protein initiators, collectively called the BMPs, which belong to the transforming growth factor β (TGF-β) super family (Betts and Sternberg, 1999).

BMPs affect no biological matrices; make them to have the capacity to induce bone, cartilage, ligament and tendon at both heterotopic and orthotropic sites. Release of such factors cause heterotopic ossification (Figure 2). In

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Abbreviations: BMPs, Bone morphogenetic proteins; **HO,** heterotopic ossification; **TGF-β,** transforming growth factor β; **MC,** monte carlo.

a b

Figure 1. Structure of 1tfg and 2tgi, inducible proteins, the effective proteins in heterotopic ossification.

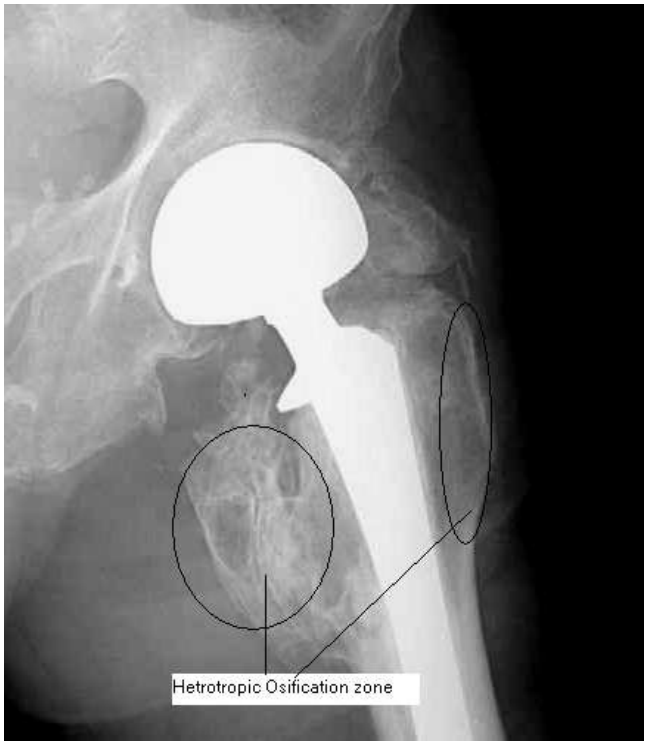

Figure. 2 Heterotopic ossification in the injury zone, cause limitation in movement.

addition, several BMPs have been shown to have a neuroprotective effect in animal models of head injury, cerebral ischemia and Parkinson's disease and may therefore have direct clinical applications for the treatment of central nervous system disorders. Our clinical trial shows that in some cases after the head trauma, the movement limitations in some skeleton regions are caused by heterotopic ossification. BMPs have a grand potential in bone formation at fracture sites and unpredicted zones. This will cause the bone hinges to be locked in an ectopic regions. Limited human studies have been performed to discuss the reasons. The action of BMP is mediated through receptor kinases and transcription factors called Smads that regulate the expression of target genes. Heterotopic ossification is a type of bone formation in the soft tissues. Soft tissue bone deposition may vary from the minimal and inconsequential to massive and clinically significant (Ruimerman, 2005).

EXPERIMENTAL

Molecular modeling methods

The modeling of biological materials requires conformational sampling. Low-energy geometries are obtained from multiple searching methods and dynamics minimizations. Mathematical methods that use random numbers for solving quantitative problems are commonly called Monte Carlo methods. Monte Carlo method solves problem by randomly selecting a large number of points within the square and determining how many of these points fall within the circle. Refereeing to steric hindrances, the main chain of a polypeptide usually has energetically favorable conformations (Ruimerman et al., 2001; Prosinecki et al., 2007). These conformations can be characterized by the value of two torsion

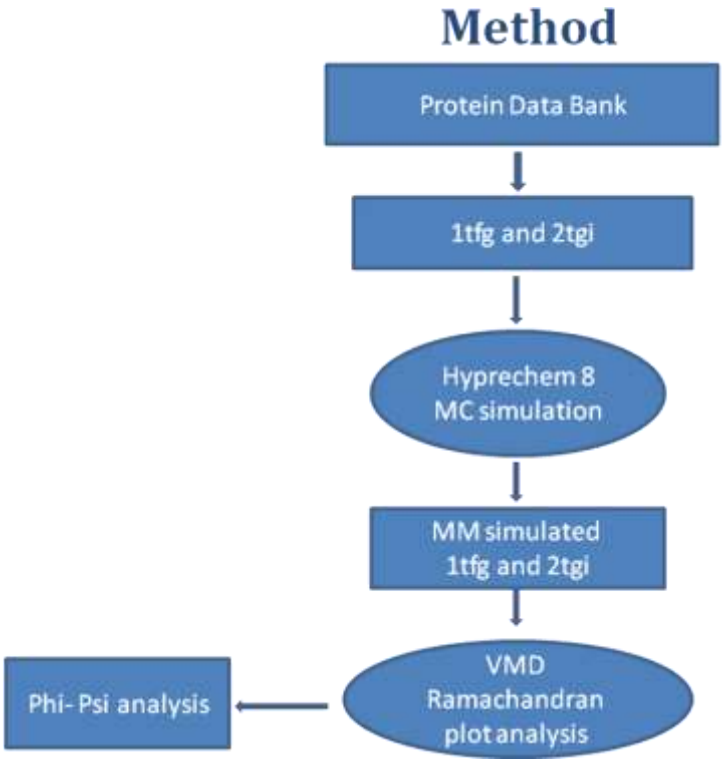

Figure.3 The computational method algorithm.

angles, φ and ψ, ω. The third angle is restricted to values of 180° for *trans*-peptide, and 0° for *cis*-peptides). The φ angle is defined by the torsion Ci–1-Ni-Cαi-Ci, and y by the torsion Ni-Cαi-Ci-Ni+1. The Ramachandran plot is defined as the distribution of φ and ψ. More than ten years ago, such plots were used to remove two structures from a database of high-resolution structures that had been created for model building in experimental maps. The Ramachandran plot is one of the simplest and most sensitive methods to assess the protein model in the absence of experimental data. It will clearly show how well the find ψ, φ angles cluster (Podtelezhnikov, 2005).
In recent study, 1 tfg and 2 tgi protein crystallography concluded pdb file selected from protein data bank as the main targets. φ and ψ of all 110 amino acid were calculated in both proteins after Montecarlo simulation with Hyperchem7.4 (2005), and the Ramachandran plot was designed, using VMD software, to compare the amino acid positions before and after Monte Carlo (MC) simulation at 290, 310 and 315 K for 200 ps Both two proteins were included sufficient water in a box around to make the simulation more real (Ruimerman, 2005; Betts and Sternberg, 1999). The conformational changes and dihedrals of φ and ψ were analyzed step by step for both proteins, as it is shown in Figure 3.

RESULTS

MC simulation were done at 290, 310 and 315 K for 200 ps. Due to the crystallographic data the 1tfg has 104/110 (94.50%) amino acid in favored region and 109/110(99.10%) of them are in allowed zone. Where, 2 tgi the 106/110 (96.40%) amino acid in favored region and 110/110 (100%) (Table 3). Increasing in temperature of Montecarlo simulation reveals the decreasing in both favored and allowed φ and ψ dihedrals for 1tfg, although some data were not obtained because of protein complicity during simulation. In 315 K, the structure of protein was not calculable by software (Tables 1 and 3). 2 tgi, shows another behavior, increasing the temperature cause the decreasing in φ and ψ dihedral angels, where increasing up to 290 K decrease the allowed dihedral and up to this temperature the allowed dihedrals decreased (Tables 2 and 3).

DISCUSSION

Nowadays, to get the injured zone of fraction warm, by different methods in Physiotherapy centers, one of the suggested methods to prevent heterotopic ossification (Ruimerman, 2005). The present study will prove the accuracy of this method, biochemically. There are two effective growth hormone, in the super family of Transforming growth factor β, 1t fg and 2 tgi. Primary studies revealed some differences between these two peptides (Figure 4). The behavior of these two factors is different in various temperatures due to the result.

CONCLUSIONS

Heterotopic ossification develops when soft tissue mesenchymal cells undergo metaplasia. This is caused

Table 1. φ and ψ dihedral analysis of 110 amino acid, 1tfg. Results were obtained by ramachandran plot, after montecarlo simulation.

		Temperature (k)								
		273 K			290			310 K		
No	Amino acid	Phi	Psi	Region	Phi	Psi	Region	Phi	Psi	Region
1	ALA	0.00	152.38	N.A.	0.00	170.92	N.A.	0.00	157.28	N.A.
2	LEU	-	-	-	-85.90	45.74	A.	-62.98	35.79	N.A.
3	ASP	-74.01	177.83	A.	-96.83	-171.98	A.	-89.05	173.43	F.
4	ALA	-43.06	-38.97	A.	-71.95	-19.39	A.	-35.37	-63.16	N.A.
5	ALA	-72.75	-22.78	A.	-	-	-	-19.92	-52.72	N.A.
6	TYR	53.93	0.00	N.A.	-103.41	-28.96	A.	-75.17	-57.02	F.
7	CYS	0.00	92.34	N.A.	-109.17	-23.49	A.	-92.88	-37.08	F.
8	PHE	89.61	-34.19	N.A.	-81.29	-12.91	N.A.	-54.96	-42.64	F.
9	ARG	-161.34	0.00	N.A.	-92.28	-9.39	N.A.	-72.49	-7.71	N.A.
10	ASN	-	-	-	-176.87	114.47	A.	171.24	80.67	N.A.
11	VAL	-179.96	165.94	A.	-70.45	122.65	F.	-32.53	103.57	N.A.
12	GLN	-	-	-	-102.08	-174.74	A.	-87.19	177.52	A.
13	ASP	-	-	-	-97.11	-1.63	N.A.	-80.70	-30.61	A.
14	ASN	-	-	-	-98.05	-176.06	A.	-61.47	167.93	F.
15	CYS	0.00	61.08	F.	-64.37	97.94	F.	-20.06	88.51	N.A.
16	CYS	-	-	-	-127.98	154.33	F.	-134.45	137.33	F.
17	LEU	-131.16	160.06	F.	-73.75	137.29	F.	-50.49	139.19	A.
18	ARG	-127.89	137.93	F.	-119.97	149.46	F.	-134.88	131.43	F.
19	PRO	-26.79	-117.11	N.A.	-81.23	121.44	F.	-68.15	123.40	F.
20	LEU	-167.52	74.91	A.	-140.48	89.94	F.	-138.69	78.75	A.
21	TYR	-21.84	-55.72	N.A.	-80.17	119.90	F.	-48.71	98.69	A.
22	ILE	-56.14	72.84	N.A.	-107.84	106.98	F.	-99.34	99.74	F.
23	ASP	-6.09	159.62	N.A.	-75.45	131.01	F.	-61.21	100.11	F.
24	PHE	137.58	108.53	N.A.	-68.22	-43.71	F.	-6.29	-76.92	N.A.
25	LYS	30.48	0.00	N.A.	-87.60	-27.70	A.	-80.66	-32.34	A.
26	ARG	-	-	-	-85.96	-20.77	A.	-84.74	-17.85	N.A.
27	ASP	-	-	-	-115.35	-22.60	N.A.	-120.94	-29.22	N.A.
28	LEU	153.80	58.02	N.A.	-115.72	-10.85	N.A.	-111.83	-4.86	N.A.
29	GLY	-142.61	-36.66	-	76.68	0.29	N.A.	53.08	15.40	N.A.
30	TRP	-108.52	145.73	F.	-74.48	117.40	F.	-82.47	121.70	F.
31	LYS	-20.03	-59.66	N.A.	-108.86	2.18	N.A.	-104.81	5.44	N.A.
32	TRP	-40.05	0.00	N.A.	-85.03	-15.41	N.A.	-90.40	-29.42	A.
33	ILE	-54.70	76.11	A.	-89.47	109.16	F.	-76.67	75.55	A.
34	HIS	-43.81	-35.32	A.	-78.12	-14.50	N.A.	-36.53	-17.33	N.A.
35	GLU	-170.14	126.09	A.	-168.68	133.06	A.	177.58	125.68	N.A.
36	PRO	-63.28	-174.76	A.	-81.30	-171.98	A.	-57.89	171.59	F.
37	LYS	-58.64	0.00	N.A.	-88.19	-1.56	N.A.	-45.37	-27.93	A.
38	GLY	140.40	177.47	N.A.	170.17	176.10	N.A.	173.05	-179.60	N.A.
39	TYR	-158.54	166.01	A.	-157.03	174.03	A.	-163.50	176.31	A.
40	ASN	-43.86	105.91	A.	-102.11	65.50	A.	-95.65	46.18	A.
41	ALA	29.18	-37.53	N.A.	-64.33	-38.18	F.	-54.01	-30.39	A.
42	ASN	-1.66	-170.94	N.A.	74.18	-173.37	N.A.	63.20	-173.02	N.A.
43	PHE	-170.14	161.52	A.	-167.54	165.96	A.	-161.33	165.22	A.
44	CYS	-	-	-	-113.31	135.43	F.	-123.62	129.96	F.
45	ALA	-	-	-	-145.36	-176.56	A.	-153.13	149.60	A.
46	GLY	-	-	-	91.31	174.32	N.A.	106.48	173.58	N.A.
47	ALA	-	-	-	-88.42	161.57	F.	-65.14	174.66	F.
48	CYS	-	-	-	-116.47	78.86	A.	-124.30	53.09	A.

Table 1. Contnd

49	PRO	-	-	-	-73.18	176.43	A.	-55.44	171.26	F.
50	TYR	-	-	-	-64.64	117.70	F.	-34.33	100.09	N.A.
51	LEU	-	-	-	73.56	12.98	N.A.	82.39	-10.63	N.A.
52	TRP	-	-	-	-86.52	83.77	A.	-65.95	90.36	A.
53	SER	76.36	-15.51	N.A.	67.79	35.40	N.A.	61.53	9.60	N.A.
54	SER	-87.11	133.68	F.	-65.09	110.39	F.	-13.40	80.75	N.A.
55	ASP	62.62	-6.93	N.A.	-68.40	-34.87	A.	-44.90	-51.48	A.
56	THR	44.62	169.25	N.A.	-133.01	179.68	A.	-125.74	189.86	F.
57	GLN	37.11	0.00	N.A.	-71.98	-23.45	A.	-58.63	-29.11	A.
58	HIS	-21.53	-58.09	N.A.	-65.09	110.39	-	-51.38	-66.65	A.
59	SER	-	-	-	-71.13	-26.10	-	-30.70	-56.92	N.A.
60	ARG	-	-	-	-83.28	-37.46	F.	-42.29	-62.92	A.
61	VAL	-	-	-	-73.43	-29.65	A.	-56.24	-37.65	A.
62	LEU	-	-	-	-86.85	-18.49	A.	-76.56	-51.05	F.
63	SER	-	-	-	-78.07	-10.69	N.A.	-37.40	-16.63	N.A.
64	LEU	-	-	-	-101.18	-31.30	A.	-111.06	-31.59	A.
65	TYR	-	-	-	-70.18	-30.71	A.	-56.05	-54.53	B-G
66	ASN	-	-	-	-75.69	-17.99	N.A.	-39.25	-58.26	N.A.
67	THR	-	-	-	-78.63	-36.16	A.	-33.50	-67.14	N.A.
68	ILE	-	-	-	-92.99	-31.16	A.	-73.67	-31.88	A.
69	ASN	-	-	-	-123.71	57.52	A.	-119.39	43.16	A.
70	PRO	-	-	-	-64.61	-13.02	N.A.	-48.57	-34.01	A.
71	GLU	-	-	-	-83.27	-4.56	N.A.	-55.12	-28.31	A.
72	ALA	-	-	-	-89.86	-13.52	N.A.	-74.37	-21.88	A.
73	SER	-	-	-	67.62	18.86	N.A.	74.67	4.58	N.A.
74	ALA	-	-	-	-92.60	29.70	A.	-57.91	-2.61	N.A.
75	SER	-	-	-	-75.37	150.21	F.	-53.50	144.88	F.
76	PRO	-	-	-	-71.51	150.78	F.	-50.58	151.92	A.
77	CYS	-	-	-	-98.92	154.69	F.	-91.91	139.27	F.
78	CYS	-	-	-	-86.61	81.50	A.	-59.36	44.04	N.A.
79	VAL	0.00	174.27	N.A.	-89.27	177.40	A.	-17.32	141.19	N.A.
80	SER	0.00	-80.76	N.A.	-78.37	143.65	F.	-51.64	130.79	A.
81	GLN	0.00	-104.73	N.A.	-109.41	-53.16	F.	-101.22	-71.56	A.
82	ASP	-85.89	150.93	F.	-113.92	141.29	F.	-77.72	140.82	F.
83	LEU	-145.89	152.31	A.	-136.06	162.76	F.	-130.23	161.52	F.
84	GLU	-132.60	137.89	F.	-133.94	155.04	F.	-127.26	143.88	F.
85	PRO	-48.67	163.32	A.	-81.31	157.20	F.	-77.63	164.70	F.
86	LEU	-144.43	131.66	F.	-136.80	126.82	F.	-128.12	132.85	F.
87	THR	-105.10	107.21	F.	-92.43	126.34	F.	-97.66	120.89	F.
88	ILE	-95.52	167.73	F.	-111.39	169.92	F.	-92.40	167.85	F.
89	LEU	-147.12	38.33	A.	-141.05	68.28	A.	-145.91	41.39	A.
90	TYR	-47.82	159.59	A.	-77.88	163.21	F.	-50.76	148.68	A.
91	TYR	-116.97	119.20	F.	-115.31	124.71	F.	-95.23	123.97	F.
92	ILE	-64.43	32.95	N.A.	-88.73	57.90	A.	-73.85	37.66	N.A.
93	GLY	84.98	-98.14	N.A.	82.14	-96.34	N.A.	82.54	-87.30	N.A.
94	LYS	-63.32	-24.91	A.	-104.22	6.76	N.A.	-107.62	-0.01	N.A.
95	THR	-90.65	105.25	F.	-100.64	114.78	F.	-92.27	113.94	F.
96	PRO	-68.97	74.25	A.	-75.11	90.15	F.	-69.48	73.12	
97	LYS	-71.27	84.12	A.	-90.88	101.87	F.	-75.35	68.76	A.
98	ILE	-45.90	109.10	A.	-84.95	131.29	F.	-27.47	124.44	N.A.
99	GLU	-106.36	168.72	F.	-124.57	166.87	F.	-123.96	157.56	F.
100	GLN	-143.57	69.30	A.	-131.32	63.76	A.	-131.97	54.28	A.

Table 1. Contnd

101	LEU	-67.59	112.59	F.	-59.32	115.32	F.	-46.34	84.93	N.A.
102	SER	-57.38	136.34	F.	-80.29	145.24	F.	-35.43	134.28	N.A.
103	ASN	90.63	5.35	N.A.	82.11	18.58	N.A.	85.98	8.25	N.A.
104	MET	-43.33	-37.81	A.	-79.29	-5.75	N.A.	-70.06	-8.45	N.A.
105	ILE	-126.02	95.16	F.	-134.36	108.95	F.	-136.90	84.32	A.
106	VAL	-69.36	111.39	F.	-80.92	116.11	F.	-63.75	109.47	F.
107	LYS	-91.20	-2.05	A.	-97.01	-9.42	N.A.	-84.65	-6.50	N.A.
108	SER	-168.77	-125.55	N.A.	-163.06	155.11	A.	-174.69	126.12	A.
109	CYS	-	-	-	-104.92	-177.57	A.	-74.53	-167.73	A.
110	LYS	0.00	0.00	N.A.	-170.52	151.30	A.	175.17	134.18	N.A.

A. =Allowed, F. = Favored, N.A. = Not allowed.

Table 2. φ and ψ dihedral analysis of 110 amino acid, 2 tgi. Results were obtained by ramachandran plot, after montecarlo simulation.

		Temperature (K)											
		273			**290**			**310**			**315**		
No	**Amino acid**	**Phi**	**Psi**	**Region**	**Phi**	**Psi**	**Region**	**Phi**	**Psi**	**Region**	**Phi**	**Psi**	**Region**
1	ALA	0.00	142.92	N.A.	0.00	153.84	N.A.	0.00	120.04	N.A.	0.00	141.21	N.A.
2	LEU	-45.23	63.25	A.	-65.04	55.48	N.A.	-55.96	50.40	N.A.	-69.14	76.42	A.
3	ASP	-119.47	-179.82	A.	-118.53	-176.72	A.	-117.15	166.27	F.	-118.25	-174.35	A.
4	ALA	-46.42	-53.01	A.	-37.11	-56.81	N.A.	-46.81	-49.01	A.	-	-	-
5	ALA	-41.73	-69.08	N.A.	-48.48	-58.34	A.	-34.10	-51.32	N.A.	-59.35	-44.68	F.
6	TYR	-46.90	-65.39	A.	-43.72	-78.45	N.A.	-50.03	-79.65	N.A.	-53.43	-51.01	A.
7	CYS	-80.12	-60.07	A.	-65.42	-53.73	F.	-72.38	-48.82	F.	-88.27	-46.98	F.
8	PHE	-29.29	157.73	N.A.	-57.84	147.42	F.	-56.56	163.51	F.	-66.86	148.45	F.
9	ARG	43.66	26.92	A.	70.84	-0.52	N.A.	56.41	18.29	A.	-	-	-
10	ASN	-136.57	130.12	F.	-116.94	141.50	F.	137.37	125.59	F.	-128.56	117.57	F.
11	VAL	-95.74	95.55	F.	-87.03	98.32	F.	-85.72	73.67	A.	-78.63	111.46	F.
12	GLN	-107.03	147.69	F.	-112.57	149.07	F.	-110.71	136.16	F.	-	-	-
13	ASP	-75.45	-30.90	A.	-	-	-	-59.81	-35.13	A.	-76.18	-31.91	A.
14	ASN	-75.49	154.34	F.	-39.46	151.07	N.A.	-71.74	152.92	F.	-35.53	151.88	N.A.
15	CYS	-12.22	101.05	N.A.	-30.07	120.39	N.A.	-31.41	114.64	N.A.	-38.18	124.96	N.A.
16	CYS	-146.74	145.41	F.	-165.57	138.18	A.	-149.28	160.59	A.	-172.82	135.62	A.
17	LEU	-57.14	130.77	F.	-24.34	151.07	N.A.	-49.03	109.58	A.	-46.51	124.95	A.
18	ARG	-121.42	137.56	F.	-119.60	132.93	F.	-105.71	128.84	F.	-112.86	142.73	F.
19	PRO	-69.66	145.97	F.	-61.73	152.84	F.	-56.22	142.21	F.	-71.46	145.14	F.
20	LEU	-158.56	97.22	A.	-172.05	80.12	A.	-162.95	94.89	A.	-161.12	102.31	A.
21	TYR	-92.39	105.93	F.	-62.55	115.73	F.	-82.24	115.82	F.	-74.63	105.92	F.
22	ILE	-91.58	106.98	F.	-102.77	95.40	F.	-90.62	108.31	F.	-73.99	117.87	F.
23	ASP	-73.19	119.18	F.	-41.33	110.01	N.A.	-70.69	107.02	F.	-85.74	107.23	F.
24	PHE	-42.04	-87.14	N.A.	-28.64	-73.07	N.A.	-34.62	-67.85	N.A.	-	-	-
25	LYS	-18.85	-80.05	N.A.	-55.84	-81.43	N.A.	-61.12	-58.83	F.	-58.64	-65.31	A.
26	ARG	-35.77	-51.32	N.A.	-16.69	-63.68	N.A.	-50.35	-47.84	A.	-40.30	-57.62	N.A.
27	ASP	-83.33	-61.16	A.	-89.28	-57.89	F.	-88.45	-48.03	F.	-87.89	-36.21	A.
28	LEU	-91.30	-23.94	A.	-70.82	-35.48	A.	-93.83	-32.46	A.	-103.59	-39.65	F.
29	GLY	92.09	1.43	N.A.	81.26	12.00	N.A.	101.73	-12.88	N.A.	79.22	24.59	N.A.
30	TRP	-55.74	72.07	N.A.	-65.56	86.08	A.	-68.99	86.61	A.	-78.61	84.94	A.
31	LYS	-55.10	-42.70	F.	-60.23	-33.72	A.	-43.24	-36.60	A.	-38.66	35.54	N.A.
32	TRP	-65.05	-28.28	A.	-72.56	-37.38	A.	-90.01	-18.24	N.A.	-81.73	33.03	A.
33	ILE	-56.50	109.28	F.	-36.39	100.39	N.A.	-57.90	117.53	F.	-74.42	109.53	F.

Table 2. Contd.

34	HIS	-68.88	-51.26	F.	-69.26	-52.00	F.	-74.42	-52.14	F.	-55.65	-69.15	A.
35	GLU	-161.14	120.79	A.	-153.98	107.42	F.	-153.98	116.77	F.	-131.14	143.49	F.
36	PRO	-60.01	174.15	F.	-79.53	-179.28	A.	-75.99	172.29	F.	-48.59	171.37	A.
37	LYS	-79.79	-25.65	A.	-57.97	-25.31	A.	-64.79	-50.18	F.	-69.11	-48.57	F.
38	GLY	-163.08	-163.50	A.	165.20	149.01	N.A.	-142.23	-145.80	N.A.	-149.12	161.97	N.A.
39	TYR	-163.80	163.36	A.	-163.76	142.39	A.	-174.99	154.44	A.	-163.82	166.82	A.
40	ASN	-94.52	60.01	A.	-52.94	68.23	N.A.	-79.10	72.61	A.	-85.92	79.19	A.
41	ALA	-32.32	-49.70	N.A.	-51.77	40.08	A.	-45.30	-54.99	A.	-51.63	-62.38	A.
42	ASN	-	-	-	60.25	-177.57	N.A.	73.26	168.38	N.A.	79.52	176.99	N.A.
43	PHE	177.68	142.10	N.A.	-155.70	152.17	A.	-157.53	151.74	A.	-161.61	165.81	A.
44	CYS	-112.90	141.87	F.	-97.65	125.50	F.	-100.04	124.44	F.	-112.79	136.18	F.
45	ALA	-164.75	118.38	A.	-139.28	158.33	F.	-154.78	126.12	F.	-159.23	143.22	A.
46	GLY	154.23	165.84	N.A.	123.33	146.86	N.A.	143.64	170.14	N.A.	117.49	149.74	N.A.
47	ALA	-42.28	142.30	N.A.	-29.32	136.09	N.A.	-52.92	138.74	A.	-11.08	144.73	N.A.
48	CYS	-106.78	73.83	A.	-109.31	75.14	A.	-110.86	58.29	A.	-131.59	63.78	A.
49	PRO	-52.57	148.87	A.	-73.18	-179.63	A.	-63.53	156.87	F.	-37.31	155.45	N.A.
50	TYR	-4.86	81.26	N.A.	-39.74	105.62	N.A.	-15.89	104.64	N.A.	-42.55	118.54	N.A.
51	LEU	87.60	-7.38	N.A.	77.41	-13.43	N.A.	80.40	-14.84	N.A.	87.42	-86.14	N.A.
52	TRP	-54.34	86.50	N.A.	-38.53	81.36	N.A.	-51.24	95.73	A.	-63.80	93.73	A.
53	SER	72.54	17.11	N.A.	58.57	16.48	N.A.	60.57	19.42	A.	63.91	16.59	N.A.
54	SER	-34.32	116.38	N.A.	-17.99	92.14	N.A.	-36.14	119.78	N.A.	-36.17	-161.97	N.A.
55	ASP	-66.90	-61.62	A.	-40.84	-84.12	N.A.	-64.99	-56.18	F.	-82.79	-56.82	F.
56	THR	-124.62	165.98	F.	-99.56	169.13	F.	-	-	-	-106.66	169.16	F.
57	GLN	-42.14	-55.07	N.A.	-26.74	-65.47	N.A.	-64.68	-41.41	F.	-	-	-
58	HIS	-34.10	-81.38	N.A.	-30.91	-62.81	N.A.	-41.86	-55.51	N.A.	-44.68	-60.37	A.
59	SER	-2.37	-74.63	N.A.	-46.66	-52.86	A.	-32.12	57.33	N.A.	-29.19	52.55	N.A.
60	ARG	-43.66	-82.76	N.A.	-44.56	-74.03	N.A.	-56.26	-71.95	A.	-57.63	-58.99	F.
61	VAL	-17.50	-64.06	N.A.	-39.59	-63.88	N.A.	-4.49	-92.01	N.A.	-43.09	-71.03	N.A.
62	LEU	-68.70	-28.21	A.	-48.65	-35.91	A.	-14.22	-60.56	N.A.	-36.97	-47.52	N.A.
63	SER	-51.34	-64.23	A.	-56.60	-55.59	F.	-50.82	-51.35	A.	-47.84	-54.33	A.
64	LEU	-24.17	-83.41	N.A.	-36.40	85.25	N.A.	-41.85	-78.45	N.A.	-48.87	-63.91	A.
65	TYR	-20.94	-67.96	N.A.	-5.18	-83.73	N.A.	-31.38	-58.12	N.A.	-34.20	-52.61	N.A.
66	ASN	-41.87	-41.66	N.A.	-28.26	-55.71	N.A.	-53.51	-35.61	A.	-59.97	-49.57	F.
67	THR	-53.27	-57.11	A.	-22.72	-72.68	N.A.	-47.60	-76.18	N.A.	-14.59	85.31	N.A.
68	ILE	-73.82	-41.46	F.	-67.62	-44.74	F.	-18.51	-61.69	N.A.	-41.67	-59.40	N.A.
69	ASN	-118.65	59.69	A.	-165.57	138.18	A.	-124.22	69.75	A.	-109.16	67.92	A.
70	PRO	-43.23	-43.04	A.	-64.18	-30.72	A.	-67.01	-40.56	F.	-49.81	-41.47	A.
71	GLU	-61.14	-27.11	A.	-58.05	-43.92	F.	-22.85	47.07	N.A.	-42.64	-41.52	N.A.
72	ALA	-24.32	-50.64	N.A.	-8.77	-49.09	N.A.	-50.72	-39.05	A.	-40.66	-41.26	N.A.
73	SER	77.88	0.45	N.A.	53.02	22.26	N.A.	64.69	37.23	N.A.	73.00	16.52	N.A.
74	ALA	-79.91	3.12	N.A.	-78.92	-1.78	N.A.	-101.46	7.21	N.A.	-96.96	7.87	N.A.
75	ALA	-35.26	146.23	N.A.	-31.22	152.25	N.A.	-53.75	128.01	A.	-43.85	129.45	A.
76	PRO	-66.49	132.17	F.	-62.64	195.05	F.	-37.05	167.91	N.A.	-35.54	146.05	N.A.
77	CYS	-95.68	133.54	F.	-110.98	132.27	F.	-139.42	131.33	F.	-90.12	133.87	F.
78	CYS	-53.33	107.15	A.	-49.74	154.02	A.	-53.14	91.50	A.	-61.68	92.21	A.
79	VAL	-131.01	147.48	F.	-137.01	152.60	F.	125.47	159.61	F.	-116.48	177.29	A.
80	SER	-32.28	144.94	N.A.	-35.54	113.71	N.A.	-43.40	119.64	A.	-49.08	148.25	A.
81	GLN	-127.46	-68.39	A.	-92.09	-80.81	N.A.	-97.51	70.67	A.	-120.59	-73.61	N.A.
82	ASP	-78.25	131.04	F.	-	-		-93.59	131.47	F.	-84.23	138.84	F.
83	LEU	-129.91	153.43	F.	-113.17	162.15	F.	-122.11	152.95	F.	-141.55	156.98	A.
84	GLU	-107.54	159.93	F.	-124.84	149.94	F.	-111.66	160.96	F.	-134.01	152.59	F.
85	PRO	-69.33	174.16	F.	-73.73	177.31	A.	-	-	-	-64.41	177.99	F.

Table 2. Contd.

86	LEU	-135.07	134.26	F.	-153.79	113.08	F.	-151.93	118.11	F.	-114.17	108.47	F.
87	THR	-80.44	126.80	F.	-66.13	125.95	F.	-61.25	117.46	F.	-48.23	114.13	A.
88	ILE	-109.81	151.76	F.	-114.74	156.91	F.	-111.32	134.68	F.	-102.63	167.55	F.
89	LEU	-129.44	115.26	F.	-121.54	122.58	F.	-100.82	132.83	F.	-144.72	122.16	F.
90	TYR	-120.07	159.99	F.	-134.98	162.06	F.	-151.14	144.99	A.	-148.68	131.31	F.
91	TYR	-92.22	131.52	F.	-58.95	140.01	F.	-72.64	139.12	F.	-54.51	129.48	F.
92	ILE	-95.07	84.87	A.	-114.30	92.84	F.	-112.46	92.43	F.	-113.41	71.00	A.
93	GLY	71.33	-92.72	N.A.	70.12	-103.27	N.A.	57.61	-114.84	N.A.	75.87	-86.14	N.A.
94	LYS	-129.01	-0.03	N.A.	-123.51	-10.49	N.A.	-107.75	-11.43	N.A.	-133.23	12.65	N.A.
95	THR	-39.42	79.15	N.A.	-59.62	94.95	A.	-53.24	100.95	A.	-85.14	95.84	F.
96	PRO	-42.46	126.35	N.A.	-50.51	108.15	A.	-50.51	143.52	A.	-46.85	120.46	A.
97	LYS	-114.30	95.58	F.	-103.02	104.10	F.	-138.71	116.81	F.	-103.43	117.95	F.
98	ILE	-59.18	130.38	F.	-86.72	138.16	F.	-108.76	110.78	F.	-85.29	119.31	F.
99	GLU	-133.62	134.63	F.	-146.43	102.17	F.	-127.50	114.77	F.	-119.25	123.34	F.
100	GLN	-87.37	109.16	F.	-70.56	98.44	F.	-43.34	105.59	A.	-95.92	86.03	A.
101	LEU	-96.80	110.51	F.	-87.17	109.91	F.	-100.41	105.76	F.	-53.02	121.06	A.
102	SER	58.11	151.25	F.	-47.77	154.02	A.	-58.25	149.18	F.	-68.37	163.48	F.
103	ASN	68.94	14.24	N.A.	62.91	28.66	N.A.	59.21	47.92	A.	64.36	18.99	N.A.
104	MET	-71.01	-41.25	F.	-79.55	-52.58	F.	-96.57	-30.54	A.	-79.01	-61.31	A.
105	ILE	-104.09	117.22	F.	-108.13	117.02	F.	-120.68	118.93	F.	-90.66	127.13	F.
106	VAL	-69.19	121.15	F.	-54.95	112.71	F.	-85.62	142.93	F.	-66.09	120.84	F.
107	LYS	-102.81	-25.04	A.	-103.53	36.50	A.	-115.32	-25.09	N.A.	101.33	-20.57	A.
108	SER	-164.80	136.03	A.	-127.19	166.96	F.	-139.97	167.84	A.	-162.90	149.01	A.
109	CYS	-119.08	166.89	F.	-132.09	162.37	F.	-111.92	157.22	F.	-125.21	-176.54	A.
110	LYS	-149.01	150.07	A.	-142.19	151.24	F.	-142.68	162.58	A.	-176.51	142.73	A.

A. =allowed, F. = favored, N.A. = not allowed.

Table 3. 1tfg and 2tgi, φ and ψ dihedral analysis after Montecarlo simulation at a glance.

		2 tgi		1 tfg	
		Favored	**Allowed**	**Favored**	**Allowed**
Crystallographic data	Ratio	106/110	110/110	104/110	109/110
	Percent	96.40%	100%	94.50%	99.10%
Simulted at 273 K	Ratio	41/110	61/110	m.d.	m.d.
	Percent	37.27%	55.45%	m.d.	m.d.
Simulted at 290 K	Ratio	40/110	60/110	38/110	78/110
	Percent	36.36%	60%	34.54%	70.90%
Simulted at 310 K	Ratio	44/110	70/110	31/110	62/110
	Percent	40%	60.64%	28.18%	58.18%
Simulted at 315 K	Ratio	34/110	74/110	m.d.	m.d.
	Percent	30.91%	67.27%	m.d.	m.d.

m.d. = missed data.

to form bone. The phenomenon is not well known. Neurogenic, traumatic and myositis ossification progressiva are the main roots of HO formation. In closed head injuries Neurogenic HO, spinal cord trauma, strokes, tumors and central nervous system infections HO has been described. It always happens after the neurologic lesion, also it may be mediated by a humoral factor. Neurogenic HO can be severe and may lead to ankylosis (Sawyer et al., 1991). This phenomenon makes some movement limitation for patients although it

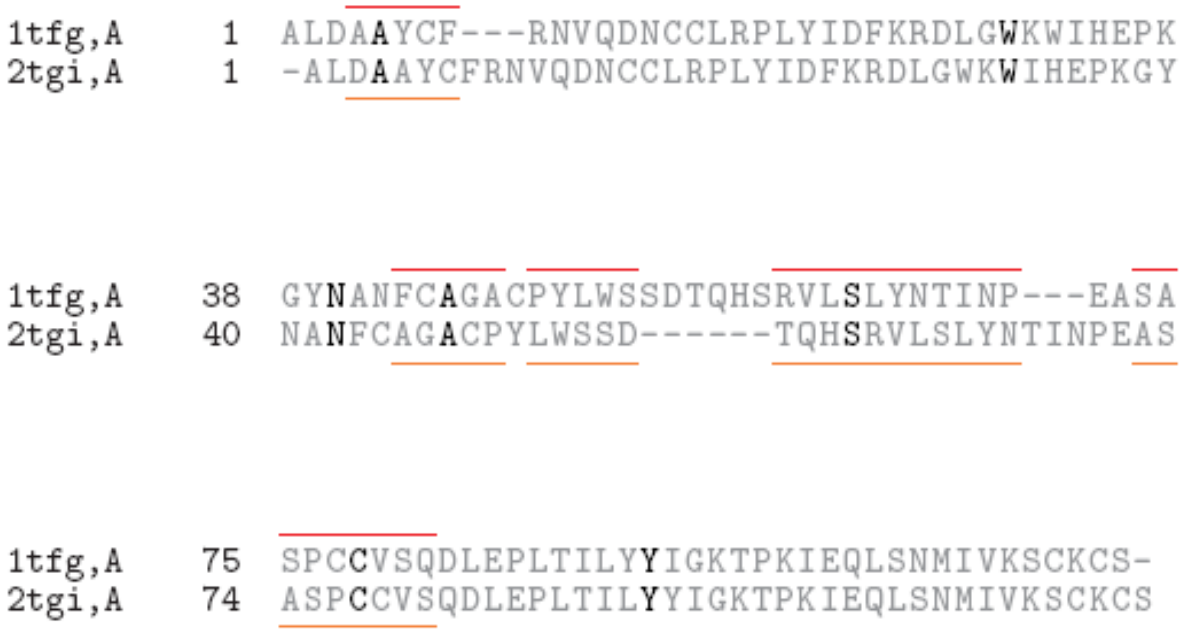

Figure. 4 Sequence comparisons of 1 tfg, and 2 tgi, data were collected from protein data bank. www.pdb.org

depends the class of HO. Both 2 tgi and 1tfg are doing inducible activities on connective tissue around the hurt zone of bone fracture, or a different zone, especially in head trauma patients which these factors elevated base on a neuroprotective role. The affected humoral factors are members of TGF- β super family. These factors are 1 tfg and 2 tgi, where the crystallographic data are available in protein data bank. Results reveal that, 1tfg is affected by increasing the temperature. The favored and allowed regions were decreased in population of dihedral angles. Therefore it is though that to keep warm the region by some equipment, with short frequencies radii, may affect this factor to inhibit its function, therefore the heterotropic ossification will decrease and it will be suggested as a role in treatment. The result is more complicated for 2tgi, increasing the temperature up to room temperature cause the protein to find more unfavored dihedral, but the more temperature cause more allowed ones. It is clear that utilization of IR lamp does not affect on such patients. Therefore it offered that in patients with head trauma, before any invasive physical therapy, the type of transforming growth factors has to be clarified biochemically.

REFERENCES

Atkinson GJ, Lee MY, Mehta MK. (2010) Heterotopic Ossification in the Residual Lower Limb in an Adult Nontraumatic Amputee Patient. Am J. Phy. Med. Rehabil., 89 (3): 245-248.

Betts MJ, Sternberg JE. (1999). An analysis of conformational changes on protein–protein association: implications for predictive docking. Protein Engineering. 12: 271-283.

HyperChem (2005). Release 7.52 Professional, Hypercube, Gainesville, FL..

Podtelezhnikov A A. (2005). Polypeptide sampling, knowledge-based potentials, and protein structure prediction, UCL, London UK.

Prosinecki V, Faísca PF, Gomes CM. (2007). Conformational States and Protein Stability from a Proteomic Perspective. Curr. Proteomics, 4: 44-52.

Ruimerman R, Huiskes R, Van Lenthe GH, Janssen JD. (2001) Bone-cell Metabolism to Mechanical Adaptation of Trabecular Architecture. Comp. Meth. BioMech. BioMed. Eng., 4:433-448

Ruimerman R. Modeling and remodeling in bone tissue (2005). Eindhoven, Netherlands.

Sanders BS, Wilcox RB, Higgins LD (2010). Heterotopic Ossification of the Deltoid.

Sawyer JR, Myers MA, Rosier RN, Puzas JE. (1991) Heterotopic ossification: Clinical and cellular aspects. Calcif Tissue Int., 49: 208-215.