대한한방내과학회지 제26권 3호(2005년 12월)
Korean J.Orient.Int. Med. 2005;26(4):753-760

항암단으로 치료한 암환자 100례의 혈청 VFGF, bEGF 및 platelet 수치 변화

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Changes of Serum VEGF, bFGF levels and platelet counts in 100 Cancer Patients treated with Hang-Am-Dan

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목적 : 본 연구는 100명의 암환자를 대상으로 항암단의 항혈관형성 효과를 측정하기 위하여 고안되었다.
방법 : 100명의 암환자 전체의 치료전후의 VEGF, bFGF 및 혈소판 수치의 변화량을 측정하였고, 병기, 삶의 질 및 암종별로 환자를 나누어 각각의 치료전후의 VEGF, bFGF 및 혈소판 수치의 변화량을 측정하여 통계적 유의성을 살펴보았다.
결과 : 항암단으로 치료한 암환자의 bFGF 수치는 치료전 후 통계적으로 유의성 있게 감소하였다. 특히 유방암 환자에서 bFGF 수치의 감소가 눈에 띄었다. 비록 통계적으로 유의하지는 않았지만 VEGF 수치도 항암단으로 치료 후 다소 감소하는 경향을 보였다.
결론 : 따라서 항암단이 암환자 치료에 있어 항혈관형성 약물로써 작용한다고 추론할 수 있다.

Key Words: Anti-Angiogenesis, Hang-Am-Dan, Vascular Endothelial Growth Factor(VEGF), Basic Fibroblast Growth Factor(bFGF), Platelet Counts

1. Introduction

Angiogenesis is the multistep formation of new blood vessels from existing vessels. It involves extracellular matrix remodeling, endothelial cell migration and proliferation, capillary differentiation, and anastomosis formation. Angiogenesis plays a very important role in physiological vascularization during the normal menstrual cycle, and it occurs in pathophysiological conditions such as wound healing, proliferative retinopathy, rheumathoid arthritis, and solid tumors[1].

Any solid tumor can only remain up to a few cubic millimeters without tumor neovasculature, and tumor angiogenesis is an essential step for further growth and metastasis[2,3]. And recently there is increasing evidence that angiogenesis may be important in malignancies[4,5].

On the other hand, angiogenesis has acquired importance as an independent prognostic indicator in solid tumors continuously[6,7]. Evaluation of circulating serum levels of angiogenic cytokines might be a possible indirect measurement of angiogenesis in

· 접수 : 2005. 8. 3. · 채택 : 2005. 8. 26.
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a non-invasive and observer-independent fashion[8,9].

Many angiogenic peptides have been identified and their clinical significance has been discussed[10]. Of these, two well-characterized peptides, vascular endothelial growth factor(VEGF) and basic fibroblast growth factor(bFGF)[10-3], are documented to play a significant role for a development of neovascularization. bFGF is a secreted multifunctional cytokine and potent stimulator of angiogenesis[14,15]. VEGF also plays an important role in inducing endothelial cell proliferation and neovascularization[16-8].

Recently, there are many clinical reports that strongly support the relevance of bFGF and VEGF as prognostic indicator for cancer patients. And previously, we presented that Hang-Am-Dan(HAD) has a anti-angiogenic properties in animal model. Therefore, we could assume that serum bFGF and VEGF levels can be used for evaluation of HAD, which have been prescribed for cancer patients in Daejeon University Oriental Hospital.

Hence, we investigated the anti-angiogenic effects of HAD by measuring the serum levels of bFGF and VEGF and platelet counts in 100 cancer patients before and after treatment with HAD.

2. Patients and Methods

2.1 Patients and drug

This retrospective study was performed on 100 cancer patients who were diagnosed as having various cancer in Korea and administered HAD in the East-West Cancer Center from November 2002 to April 2004. All patients recruited for this study were selected randomly. For further informations about the patients, we examined our medical record charts and interviewed performance status in order to get the informations about clinical coarse. We also examined the relations of 3 factors with changes of VEGF, bFGF and platelet counts in cancer patients. The criteria of this study were as follows; Sera were drawn from all patients before and after treatment and clinical informations were extracted from the clinical records. Clinical staging should be performed according to the criteria of the Union Internationale Contre le Cancer (UICC) from 1997. Performance status should be performed according to the criteria of the Eastern Cooperative Oncology Group (ECOG) from 1982. Alternatively, we didn't strictly care the medical situation with which some patients randomly had received western conven-

Table 1. Prescription of HAD

Scientific name	Relative amount(㎎)	Voucher specimen
Coicis Semen	129.5	CL-2003-01-Se
Pseudoginseng Radix	43.0	PN-2003-01-Ra
Hippocampus	13.0	HK-2003-01
Cordyceps Militaris	13.0	CM-2003-01
Santsigu Tuber	13.0	CA-2003-01-Tu
Ginseng Radix	13.0	PG-2003-01-Ra
Bovis Calculus	8.5	CB-2003-01-Ca
Margarita	8.5	PM-2003-01-Ma
Moschus	8.5	MO-2003-01
Total amount(1 capsule)	250.0	

tional treatments, such as chemotherapy or radiotherapy.

Patients continued at the 1500mg/day/person dose with no changes. HAD was offered from Daejeon Oriental Medical Hospital. Voucher specimens have been deposited at the Institute of Traditional Medicine and Bioscience in Daejeon University. The composition of HAD was listed in Table 1.

2.2. Measurement of VEGF and bFGF

Sera were collected from clotted blood followed by centrifugation (1,600g), aliquoted and stored at -70℃ until used. Serum concentrations of VEGF, bFGF were measured with Quantikine human immunoassay kits (Quantikine TM human VEGF and Quantikine TM HS human FGF basic, R&D Systems, Minneapolis, MN, USA).

2.3. Stastical analysis

Serum VEGF, bFGF concentrations and platelets counts were presented as mean ± standard deviation and changes of these values after treatment with HAD were analyzed using paired t-test. A p-value less than 0.05 was considered to indicate a statistically significant change.

3. RESULTS

3.1. Demographic and clinical characteristics

One hundred patients, 59 females and 41 males fulfilled the inclusion criteria. The mean age ± standard deviation was 51.83±11.28 years (range 18-79 age). Stage distribution was stage II in 34 patients, stage III in 28 patients and stage IV in 38 patients. Patients presented as having a performance status of 1 in 52 patients, status of 2 in 38 patients, status of 3 in 8 and status of 4 in 2 patients. The exact clinical and pathological stage distribution of all cancer patients is shown in Table 2.

Table 2. Characteristics of Patients

	No
Gender	
male	41
female	59
age(years)	
<40	10
40-49	34
50-59	32
≥60	24
Performance status	
1	52
2	38
3	8
4	2
Stage (UICC)	
II	34
III	28
IV	38
cancer portion	
breast	26
colon&rectum	18
liver	8
lung	11
pancreas	1
stomach	15
uterus cervix	4
others	17

3.2. Changes of VEGF, bFGF levels and platele counts

The mean bFGF level ± standard deviation fo the group of 73 patients decreased with treatmer from 10.5±13.54 pg/ml to 7.13±9.42 pg/ml. Seru bFGF concentrations were significantly reduced aft the treatment with HAD. And there was somewh decreasing change in VEGF levels, though the chan was not statistically significant. Platelet counts h

no changes after treatment with HAD. The changes of VEGF, bFGF levels and platelet counts are shown in Table 3.

Table 3. Changes of VEGF, bFGF levels and platelet counts

	mean±SD	P-Value
VEGF (pg/ml)(93)		0.50
Before treatment	355.4±354.3	
After treatment	333.0±281.5	
bFGF (pg/ml)(73)		0.04*
Before treatment	10.5±13.54	
After treatment	7.14±9.43	
Platelet (104/㎕)(83)		0.61
Before treatment	23.6±7.84	
After treatment	23.9±9.26	

( ): number of patients. *P<0.05

3.3. Changes of VFGF, bEGF levels and platelet counts according to the clinical stages

Serum bFGF levels of stage Ⅱ and Ⅳ patients were somewhat reduced after treatment. The changes of VEGF, bFGF levels and platelet counts according to the different clinical stages are shown in Tables 4.

Table 4. Changes of VEGF, bFGF levels and platelet counts according to the clinical stages

	mean±SD	P-Value
VEGF (pg/ml)		
StageⅡ(34)		0.23
Before treatment	387.9±289.7	
After treatment	343.0±241.3	
StageⅢ(25)		0.98
Before treatment	261.7±259.1	
After treatment	260.7±296.2	
StageⅣ(34)		0.84
Before treatment	391.7±456.2	
After treatment	376.1±304.8	
bFGF (pg/ml)		
StageⅡ(21)		0.08
Before treatment	10.7±10.7	
After treatment	7.2±10.8	
StageⅢ(25)		0.91
Before treatment	6.4±6.0	
After treatment	6.2±7.8	
StageⅣ(27)		0.10
Before treatment	14.3±18.8	
After treatment	8.0±9.9	
Platelet (104/㎕)		
StageⅡ(29)		0.65
Before treatment	25.5±7.6	
After treatment	25.0±9.2	
StageⅢ(23)		0.15
Before treatment	22.2±8.2	
After treatment	20.7±7.7	
StageⅣ(31)		0.11
Before treatment	22.8±7.6	
After treatment	25.3±10.0	

( ): number of patients.

3.4. Changes of VEGF, bFGF levels and platelet counts according to the performance status

After HAD treatment, serum bFGF levels in the performance grade 1 groups were decreased significantly from 9.9±14.2 pg/ml to 4.3±6.1 pg/ml. The changes of VEGF, bFGF levels and platelet counts according to the performance status are shown in Table 5.

Table 5. Changes of VEGF, bFGF and platelet levels according to the performance status.

	mean±SD	P-Value
VEGF (pg/ml)		
performance status of 1(49)		0.12
Before treatment	330.6±408.6	
After treatment	247.1±199.0	
performance status of 2(37)		0.37
Before treatment	403.8±297.4	
After treatment	438.9±348.8	
performance status of 3(6)		0.46
Before treatment	288.3±188.4	
After treatment	390.4±205.7	
performance status of 4(1)		
Before treatment	183.9	
After treatment	282.8	

	mean±SD	P-Value
bFGF (pg/ml)		
performance status of 1(39)		0.03*
Before treatment	9.9±14.2	
After treatment	4.3±6.1	
performance status of 2(26)		0.48
Before treatment	12.2±14.3	
After treatment	10.4±11.7	
performance status of 3(7)		0.49
Before treatment	8.4±7.6	
After treatment	11.4±11.7	
performance status of 4(1)		
Before treatment	8.0	
After treatment	1.2	
Platelet (104/㎕)		
performance status of 1(42)		0.20
Before treatment	23.9±6.8	
After treatment	22.8±8.7	
performance status of 2(32)		0.11
Before treatment	23.2±7.8	
After treatment	25.0±7.1	
performance status of 3(7)		0.97
Before treatment	24.1±12.0	
After treatment	24.3±11.7	
performance status of 4(2)		0.59
Before treatment	21.0±19.1	
After treatment	30.4±36.7	

( ): number of patients. *P<0.05

3.5. Changes of VEGF, bFGF levels and platelet counts according to the cancer portion

In uterus cervix cancer, VEGF levels were reduced significantly from 559.0±91.2 to 244.6±139.9. bFGF levels showed significant change in breast cancer. The changes of VEGF, b-FGF and platelet count according to the cancer portion are shown in Tables 6.

Table 6. Changes of VEGF, bFGF levels and platelet counts according to the cancer portion

	mean±SD	P-Value
VEGF (pg/ml)		
breast(26)		0.23
Before treatment	338.1±485.5	
After treatment	228.6±169.5	
colon&rectum(17)		0.42
Before treatment	303.7±339.4	
After treatment	350.8±289.9	
liver(7)		0.29
Before treatment	222.7±123.8	
After treatment	279.2±122.6	
lung(10)		0.55
Before treatment	395.2±303.8	
After treatment	457.3±407.3	
others(15)		0.33
Before treatment	416.4±292.6	
After treatment	489.2±397.4	
pancreas(1)		
Before treatment	313.5	
After treatment	242.1	
stomach(13)		0.25
Before treatment	368.5±322.3	
After treatment	305.8±203.5	
uterus cervix(4)		0.01*
Before treatment	559.0±91.2	
After treatment	244.6±139.9	
bFGF (pg/ml)		
breast(20)		0.04*
Before treatment	14.1±20.8	
After treatment	4.4±5.7	
colon&rectum(14)		0.27
Before treatment	9.8±10.7	
After treatment	7.7±13.3	
liver(6)		0.91
Before treatment	6.3±5.2	
After treatment	6.7±7.3	
lung(9)		0.32
Before treatment	14.8±12.7	
After treatment	9.5±8.0	
others(10)		0.79
Before treatment	5.9±5.7	
After treatment	7.0±8.3	
pancreas(1)		
Before treatment	0	
After treatment	0	
stomach(11)		0.85
Before treatment	10.1±9.1	
After treatment	10.5±12.9	
uterus cervix(2)		0.55
Before treatment	5.2±7.3	
After treatment	6.4±5.3	

	mean±SD	P-Value
Platelet ($10^4/\mu\ell$)		
breast(23)		0.80
Before treatment	21.0±6.4	
After treatment	21.4±6.4	
colon&rectum(13)		0.15
Before treatment	29.2±8.2	
After treatment	32.1±12.4	
liver(7)		0.02*
Before treatment	21.1±9.9	
After treatment	17.3±8.3	
lung(11)		0.11
Before treatment	22.8±5.7	
After treatment	20.3±5.6	
others(14)		0.45
Before treatment	23.8±10.1	
After treatment	25.3±9.6	
stomach(14)		0.62
Before treatment	23.5±5.0	
After treatment	24.4±7.2	
uterus cervix(1)		
Before treatment	32.8	
After treatment	37.3	

( ): number of patients. *P<0.05

4. Discussion

In general, VEGF and bFGF are regarded as the most potent angiogenic factors. VEGF is an endothelial cell mitogen and permeability factor that is potently angiogenic. bFGF belongs to the family of heparin-binding growth factors. It is known to induce chemotactic, angiogenic and mitogenic activity and play an important role in early differentiation and developmental processes.

Therefore, the trend of various therapeutic approaches aimed to investigate inhibiting the function of VEGF and bFGF.

Recently, many people has concerned about herbal medicine in medical research for cancer. The antitumor activity of various herbal plants have been experimented extensively and reported over the world.

Among the herbal medicine, HAD is a prescription which have been given for cancer patients in Oriental Hospital of Daejeon University since 1998. In the previous study, various clinical trials and laboratory findings assured us to believe that HAD has significant positive effects on cancer treatment.

In this study, to investigate the effect of HAD on anti-angiogenic activity, we analyzed the changes of serum VEGF, bFGF levels and platelet counts in 100 cancer patients before and after treatment with HAD.

At the start, we have observed changes between serum VEGF and bFGF levels and various variances (clinical stage, performance status and cancer portion). No significant associations were observed between levels of serum VEGF and bFGF levels and each factors. In general, previous studies showed no statistically significant differences in histology, gender, age, pathological stage with respect to serum VEGF and bFGF. Our findings did not show any correlation with each factors, too. However, some results showed statistically significant differences in performance status and cancer portion partly. For example, serum bFGF levels in the performance grade 1 groups were decreased significantly. In uterus cervix cancer, VEGF levels were reduced significantly and bFGF levels showed significant change in breast cancer. From above results, we can suppose that HAD is more effective in uterus cervix and breast cancer. Moreover, HAD has predominant effect in good performance status.

It is reported that serum VEGF levels increase during clotting as a result of its release from platelets, and plasma sample instead of serum was recommended for measuring the circulating VEGF more accurately. However, platelets have been implicated in tumor metastasis since circulating tumor cells forming aggregates with platelets were observed. Hence, to exclude the change of serum VEGF levels are affected by blood platelets, we examined the change of serum platelet counts. Our findings

did not showed statistically significant differences in the change of serum platelet counts except for liver cancer. Therefore, we supposed that it can be excluded the change of serum VEGF levels are affected by blood platelets from our findings.

In the change of serum bFGF level after HAD treatment, it was reduced significantly. Even though it wasn't statistically significant, serum VEGF concentrations decreased, too. From above the results, we can conclude that HAD may present the anti-cancer effects by anti-angiogenic response specific to cancer. However, Some methodological limitations of our study should be exhibited. 1) These results are out of place as for being generalized because the sample under investigation is too small, and results might apply only to Korea. 2) Various cancer patients in our study represent a heterogeneous group. Moreover, our study is a small one of retrospective series, thus making conclusion can be a jump in the logic. This study is needed for more accurate estimation with various variance and the administration period should be controlled more strictly.

Taken together, HAD can be a useful anticancer agent and more needs to be learned about their mechanisms of action and therapeutic potential and more clinical trials should be expected.

5. conclusion

This study was aimed to investigate the anti-angiogenic effects of HAD

1. Serum bFGF levels were reduced significantly after treatment with HAD from 10.54±13.54 to 7.14±9.43.
2. Especially in breast cancer and performance status of 1, there were significant change of serum bFGF levels.
3. Even though it wasn't statistically significant, serum VEGF levels were reduced after treatment with HAD.

We suggest that HAD has anti-angiogenic effects in cancer patients

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