Competitive Inhibition Relationship Between Erythropoietin And Interferon-Gamma In Renal Failure Anemia.

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الخلاصة

أجريت هذه الدراسة التجريبية محاولة لأثبات العلاقة التثبيطية التنافسية بين الأنترفيرون-جاما وهرمون الأرثروبويتين في حالة عجز الكلى المزمن من خلال برهنة وجود علاقة عكسية متمثلة بتثبيط الأرثروبويتين لأفراز الأنترفيرون-جاما من قبل الخلايا أحادية النوى خارج جسم الكائن الحي . حيث تم عزل هذه الخلايا من عينات الدم المأخوذة من تسعة مرضى مصابين بعجز الكلي المزمن و تسعة أشخاص أصحاء، ثم حضنت في ظروف قياسية مع المشطر فقط أو بأضافة الأرثروبويتين ألى الخليط مرة أخرى. بعد ذلك تم قياس مستوى الأنترفيرون-جاما المفرز بطريقة الفحص المناعي (ELISA).بينت النتائج أن الأرثروبويتين يثبط أفراز الأنترفيرون-جاما بشكل معنوي لدى الأشخاص الأصحاء أقل منه لدى مرضى عجز الكلى المزمن.

Abstract

This is an in vitro laboratory study. It was done in order to prove the competitive inhibition between interferon-gamma (IFN-gamma) and erythropoietin (EPO) in chronic renal failure (CRF) by fixing an inhibition elicited by EPO on IFN-gamma expression by mononuclear cells. These cells were already separated from fresh anticoaggulated blood specimens from nine CRF-patients and nine healthy persons.Then, were co incubated separately with mitogen, phytohemaggluttinin with and without EPO under standard incubation conditions. Thereafter, IFN-gamma level was measured by ELISA. Results have shown that EPO significantly inhibits IFN-gamma expression by mononuclear cells in healthy persons, p value < 0.05, while this inhibition is not significant in CRF-patients.

Introduction

Anemia is a universal complication in CRF. It has multiple causes; the most important of which is decreased production of EPO by the kidney. The availability of this hormone is revolutionizing treatment of this form of anemia, but unfortunately these patients usually develop EPO resistance mainly due to elevated erythropoiesis-inhibitory cytokines levels (1).

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Chronic inflammation and immune system activation underlie the resistance to EPO in CRF patients reflected by abnormally raised proinflammatory cytokines including IFN-gamma which are known to inhibit erythropoiesis (2).

Erythropoietin resistance may be explained by many mechanisms including the competitive inhibition by these cytokines on EPO (3) and this what we tried to improve by searching for a reverse inhibitory effect elicited by EPO on IFN-gamma.

Materials and Methods

Patients and control: Nine CRF-patients were recruited during December 2005 from Dialysis Unit in Al-Hussein Hospital in Kerbala and nine age- and gender-matched healthy persons were chosen randomly.

Specimens collection: Venous blood specimens (1 mL) were aspirated from each member of the test and control groups and put in sterile EDTA-tubes for further processing.

Mononuclear cells separation: Peripheral blood mononuclear cells (PBMCs) were separated from fresh blood specimens by density-gradient centrifugation over Ficole-Hypaque (Flow laboratories) and put in RPMI (Eurolone) separately to form (18) pools each with nearly equal cell count

Samples preparation: From each PBMC pool two samples were made as follows:-

1-First sample:- PBMCs were incubated with phytohemagglutttinine (PHA) (BDH, England) 0.3 microgram/mL in RPMI at 37 degree centigrade and 5% CO2 for 24 hours and this was regarded as the base level of IFN-gamma; one for each of the eighteen tested members.

2-Second sample:- in which PBMCs from each member were co-incubated with PHA and erythropoietin (EPO) (Eprex, Switzerland) (25 micro liters of 100 U/mL solution) under the same conditions.

Detection of interferon-gamma (IFN-gamma) level: After 24 hours of incubation, IFN-gamma level was measured in each of the 36 sample supernatants by ELISA method according to the procedure enclosed with the kit (10).

Statistical Analysis: Unpaired T-test of the two sample means at confidence interval of 5% was employed to test for any significant difference between CRF-patients and the control group in terms of the mean of base level IFN-gamma, differences between the stimulation level of IFN-gamma (secreted in response to PHA) and level of IFN-gamma expressed in the presence of PHA and EPO.

Results

The results showed that there is significant increased expression of IFN-gamma by T-cells from CRF-patients (base level IFN-gamma) compared to that from healthy individuals (mean IFN-gamma level of 155 pg/mL , versus 11.37 pg/mL in healthy individuals, p value = 0.0001) , (Table 1).

Also it was shown that EPO significantly inhibits IFN-gamma expression by T-cells and NK cells from healthy persons (mean IFN-gamma level = 69.34 pg/mL without EPO, versus 14.63 pg/mL with EPO, p value = 0.001), (Table 2). but this inhibitory effect was shown to be not significant in CRF-patients' samples in which mean IFN-gamma level was 168.72 pg/mL without EPO, versus 159.15 pg/mL with EPO, p value =2.13, (Table 3).

Table-1: IFN-gamma levels (pg/mL) detected by ELISA test that expressed in vitro by PBMCs from nine CRF-patients compared with those from control after 24-hour incubation in RPMI and phytohemaggluttinine.

	Baseline IFN-gamma levels (pg/mL)	
Samples number	CRF-patients	Healthy control
1	93.23	6.48
2	160.12	12.71
3	178.63	6.73
4	200.21	9.48
5	153.96	12.42
6	155.56	14.23
7	154.22	16.75
8	150.23	14.63
9	184.88	8.91
Mean	155	11.37
Standard error	28.51	3.63
p value for the two means =0.0001		

-IFN: interferon, PBMCs: peripheral blood mononuclear cells, CRF: chronic renal failure, cRPMI: complete RPMI; lymphocyte culture medium.

Table-2:IFN-gamma levels expressed by PBMCs from nine healthy individuals after 24-houre incubation in RPMI and phytohemaggluttinine with and without EPO.

	IFN-gamma levels picogram/mL	
Samples	Stimulation level without EPO	Stimulation level with EPO
1	8.12	9.24
2	92.43	13.46
3	92.11	15.61
4	14.71	9.47
5	39.72	13.51
6	94.81	15.67
7	92.92	20.53
8	94.51	15.12
9	94.79	19.1
Means	69.34	14.63
Standard error	37.32	3.79
p value for the two means = 0.001		

-IFN: interferon, PBMCs: peripheral blood mononuclear cells, EPO: erythropoietin.

Table-3: IFN-gamma levels from nine CRF-patients secreted byT-cells and NK-cells incubated in RPMI and phytohemaggluttinine with and without EPO in the culture media.

	IFN-gamma levels, picogram/mL (pg/mL)	
Samples	Stimulation levels without EPO	Stimulation levels with EPO
1	135.71	133.23
2	167.51	164.07
3	183.22	158.18
4	200.38	188.22
5	161.84	158.38
6	159.58	153.22
7	175.34	158.92
8	154.78	153.89
9	180.15	164.27
Means	168.72	159.15
Standard error	18.71	14.28
p value for the two means = 2.13		

-IFN: interferon, PBMCs: peripheral blood mononuclear cells, CRF: chronic renal failure, EPO: erythropoietin

Discussion

This study has shown that T-cells and NK cells (within PBMCs) from CRF-patients tend to express IFN-gamma in vitro in much higher level than those from healthy individuals(more than 10-fold increase)as was shown by some other in vitro and in vivo studies (3,4) that demonstrated an increased expression of IFN-gamma and some other cytokines in these patients. And that this increased level is a consequence of chronic inflammatory process represented by uremia (3,12,13) and may contribute, in part, to EPO-resistant anemia in some of CRF-patients.

Most in vivo and in vitro studies that have dealt with the relation between IFN-gamma and EPO demonstrated an inhibitory effect elicited by IFN-gamma on EPO or erythropoiesis (17,14,18,21), but a minority of such studies revealed that IFN-gamma has a synergistic effect with EPO on erythropoiesis (12,19), however the reverse effect (i.e.) that elicited by EPO on IFN-gamma was dealt with by a very limited aspect.

Many studies proved that IFN-gamma antagonizes the antiapoptotic effect of EPO via different mechanisms of which the competitive inhibition is the main explanation (3,4,5). This theory was reinforced by the current study by finding of inhibition of IFN-gamma expression in vitro by EPO.

Specific receptors for IFN-gamma on erythroid progenitor cells may further explain this EPO-resistant anemia. Specific binding of IFN-gamma to high affinity receptors on human erythroid colony-forming cells (5).

Despite that the exact mechanism of this anemia is not yet clear (20), the elevated IFN-gamma (and some other erythropoiesis-inhibitory cytokines) level might lead to down regulation of EPO receptors (4,5,11,14,16),compete with EPO for some post-receptor molecules; such as STAT-1alpha which was shown to be common and basic for signal transduction for both EPO and IFN-gamma (6,7,15), or might antagonize the antiapoptotic effect of EPO on erythroid progenitor cells, thus inhibiting erythropoiesis (3,8,9,12).

Recommendation: It is recommended to increase the doses of EPO (within the safe level) in treatment of EPO-resistant renal failure anemia to counteract the competetive inhibitory effect of the highly expressed IFN-gamma and other erythropoiesis-inhibitory cytokines.

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