Original Article

Effects of garlic *(Allium sativum)* extract on the expression of receptor for advanced glycation end products and proinflammatory cytokines secretion in peripheral blood mononuclear cells from patients with type 2 diabetes mellitus

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Abstract

Background: Receptor for advanced glycation end products (RAGE) plays a causative role in diabetes. Garlic (Allium sativum) belongs to compounds with anti-glycation activity that can be considered as probable therapeutic approaches in delaying or preventing the onset of diabetes complications. The aim of this study was to evaluate the influence of garlic on the RAGE expression and proinflammatory cytokines secretion in peripheral blood mononuclear cells (PBMCs) from patients with type 2 diabetes mellitus. **Materials and Methods:** The PBMCs were isolated from 20 patients with fasting blood sugar level above 126 mg/dl and treated with R10 fraction and whole garlic extract in presence or absence of glycated albumin. The expression of RAGE was detected using flow cytometry and the proinflammatory cytokines secretion was evaluated by ELISA. **Results:** Glycated albumin increased RAGE expression and proinflammatory cytokines secretion. Treatment with whole garlic extract significantly reduced TNF-α and IL-1β secretion and RAGE expression by PBMCs but R10 fraction augmented the proinflammatory cytokines and RAGE expression in absence or presence of glycated albumin. **Conclusion:** Downregulation of RAGE expression was associated with decreased secretion of IL-1β and TNF-α from PBMCs after treatment with whole garlic, while R10 fraction of garlic significantly augmented RAGE expression and proinflammatory cytokines secretion. These data indicates that modulation of RAGE expression may be one plausible reason for the garlic effects on proinflammatory cytokines secretion.

Keywords: Type 2 diabetes, Garlic extract, RAGE, advanced glycation end product, Proinflammatory cytokines

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Introduction

The receptor for advanced glycation end-products (RAGE), a multi-ligand receptor of the immunoglobulin superfamily, has been originally described as possessing a pivotal role in inflammation,

atherosclerosis, nephropathy, neurodegeneration, cancer, diabetes and its complications [1-3]. Increased expression of both RAGE and its ligands have been reported in a range of inflammatory disorders such as inflammatory bowel disease, rheumatoid arthritis, atherosclerosis, amyloidosis and the vascular complications of diabetes [4].

The multi-ligand nature of RAGE is highlighted by its ability to bind a number of ligands such as advanced glycation end-products (AGEs), high mobility group box-1 (HMGB1, amphoterin), β-amyloid fibrils, certain S100/calgranulin proteins, DNA and RNA [5-7]. AGEs include reactive, cross-linking molecules that hyperglycemia, are formed from the non-enzymatic reaction between reducing sugars and the amino groups of proteins, lipids and nucleic acids under prolonged hyperglycemia [8, 9].

Clues to principle roles for RAGE in the inflammatory response were deduced from the tissue distribution of RAGE. RAGE is expressed in monocytes/macrophages [10], T and B lymphocytes [11], dendritic cells [12] and microglia. In addition to direct stimulation of RAGE on these cells, it is evident that inflammatory cells following activation, release HMGB1 and S100/calgranulins, thereby propagating inflammatory responses at least in part via RAGE [13-15].

Evidence shows that RAGE-ligand interaction in monocytes, macrophages and microglia perpetuates inflammation by promoting phagocyte infiltration and the release of proinflammatory cytokines and chemokines [16, 17].

Nutritional compounds can be considered as possible cost-effective, non-toxic candidates for delaying or preventing complications of diabetes [18, 19]. Pharmacological approaches aimed at abolishing adverse actions of AGEs involve agents that can either; prevent AGEs formation, break existing cross-links, or block related receptors [20].

Garlic (Allium sativum) is a popular spice for cooking and one of the first recorded herbal remedies that has a long history of medicinal use for a wide variety of health problems in humans, including infection, certain type of cancers, and cardiovascular disease. The use of garlic as a folk medicine for diabetes has been reported in Europe, in India, and in the Middle East [21].

Several evidence reports the anti-inflammatory and therapeutic effects of garlic on the diabetic complications, however the underlying mechanisms are not fully addressed.

This study was conducted to determine whether garlic extract has impact on RAGE expression and proinflammatory cytokines secretion by PBMCs from patients with of type 2 diabetes mellitus (T2DM).

Methods

Patients. From December 2011 to June 2012, a total of twenty T2DM patients either men or women (aged of 58 ± 18 years) who referred to the Ganjavian Hospital of Dezful (a city in Khuzestan province located in south-west of Iran) was enrolled into the study.

The patients with T2DM were confirmed by specialist physicians according to the clinical finding and American Diabetes Association criteria [22, 23]. Patients with gastrointestinal disease, liver disease, infection, or serious complications of diabetes were excluded from study and the informed written consent was obtained from all participants prior to study. The venous blood samples were collected from all patients after overnight fasting. The study was evaluated and approved by the Ethics Committee of Dezful University of Medical Sciences.

Garlic extract preparation. The fresh garlic was purchased and extracted by a method described previously [24]. Briefly, dry garlic bulbs were peeled and homogenized with two parts of distilled water in a blender. The homogenate was filtered under vacuum through a Whatman paper and the filtrate was centrifuged at 5000 rpm for 20 min. The clear supernatant was sterilized through Millipore filter, aliquoted and stored at 4°C, until used. Whole garlic extract was run through Amicon ultrafiltration using different membranes includes 300, 100, 50, 30 and 10 pm. The ultrafiltrates were collected as residues (R) 300, 100, 50, 30 and 10. R10 was further purified by Sephadex G75.

Isolation and stimulation of peripheral blood mononuclear cells, monocytes and lymphocytes. PBMCs was isolated from 10 ml heparinized (200 IU/ml) (Sigma Chemical Co, Munich, Germany) blood using Ficoll-hypaque (Sigma Chemical Co, Munich, Germany) gradient centrifugation. The cells (5×10^6

cells/mL) were washed 3 times with PBS, resuspended in RPMI 1640 media (Gibco), supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco), and cultured at 37°C in a humidified 5% CO_2 incubator.

Monocytes and lymphocytes were isolated from PBMC using a miniMACS separation unit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's protocol. Briefly, PBMCs were incubated with MACS CD14 MicroBeads (cat. no. 130-050-201) to label the cells magnetically. Subsequently, separation was performed with the miniMACS separator unit. The magnetic cell sorting procedure was strictly performed at 4°C according to the supplier's protocol. The obtained positive (monocytes) and negative (lymphocytes) cell fractions were analysed by flow cytometry.

The separated monocytes and lymphocytes were washed 3 times with PBS, resuspended in RPMI 1640 media (Gibco), supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco), and cultured at 37°C in a humidified 5% CO2 incubator and divided in 96-well microplate at density of 2×10^5 cells/well. PBMCs, monocytes and lymphocytes from each patient were stimulated using 20µl of various concentrations (10 and 100 µg/ml) of whole garlic extract and R10 fraction in the presence or absence of glycated serum albumin (100 µg/ml) for 48h.

The measurement of TNF-α and IL-1β secretion. After the incubation of PBMCs, monocytes and lymphocytes with various concentrations of whole garlic extract and R10 fraction in the presence or absence of glycated serum albumin for 48h, the supernatant was aspirated and the TNF-α and IL-1β levels were measured using sandwich ELISA (Quantikine, R&D Systems, Minneapolis, MN).

Flow cytometric analysis. For detection of surface expression of RAGE, PBMCs, monocytes and lymphocytes were incubated with mouse FITC-conjugated anti-human RAGE (1µg/10^6 cells/100µl, 10min/4ºC) antibody or with isotype control mouse IgG1 (Santa Cruz, CA) After two times washing, these were incubated with 1 µg/10^6 cells/100µl, 10 min/4ºC of FITC-conjugated polyclonal goat anti-mouse immunoglobulins (DakoCytomation). Then the cells were washed, resuspended in 500 µl PBS and analyzed with a FACScan cytometer (Becton and Dickinson, San Jose, CA).

Statistical analysis. Statistical analyses were performed by SPSS (version 19). We used the one way ANOVA and student t-test to show differences between TNF-α and IL-1β secretion and RAGE expression in stimulated cells compared to controls. The results are presented as mean±standard deviation.

Results

The effects of whole garlic extract on RAGE expression in PBMCs, monocytes and lymphocytes. As demonstrated in Table1 and fig. 1, the mean levels of the RAGE expression by PBMCs in the presence of glycated serum albumin, whole garlic extract (10 or 100 µg/ml) plus glycated serum albumin were significantly higher as compared with PBMCs from control culture (P<0.001, P<0.001, P<0.001, respectively). Although, 10 µg/ml dose of whole garlic extract could not decrease the RAGE expression by PBMCs , the mean levels of the RAGE expression by PBMCs incubated in the presence of 100 µg/ml whole garlic extract were significantly lower as compared to PBMCs from control culture (P<0.002). The mean levels of the RAGE expression by PBMCs incubated in the presence of 100 µg/ml whole garlic extract plus glycated serum albumin were also significantly lower as compared to PBMCs incubated in the presence of 10 µg/ml whole garlic extract plus glycated serum albumin (P<0.001). Moreover, the mean levels of the RAGE expression by PBMCs incubated in the presence of 10 µg/ml whole garlic extract plus glycated serum albumin was significantly lower in comparison with PBMCs incubated in the presence of only glycated serum albumin (P<0.02).

The mean levels of the RAGE expression by monocytes in the presence of glycated serum albumin, whole garlic extract (10 or 100 µg/ml) plus glycated serum albumin, were significantly higher as compared with monocytes from control culture (P<0.001, P<0.001 and P<0.001, respectively). No significant difference was observed between 10µg and 100µg dose of whole garlic extract regarding the mean levels of the RAGE expression by monocytes. The mean levels of the RAGE expression by monocytes incubated in the presence of 100 µg dose whole garlic extract plus glycated serum

Table 1: Evaluation of RAGE expression on PBMCs, monocytes and lymphocytes of diabetic patients by flowcytometry before and after the effect of glycated serum albumin, whole garlic extract and its R10 fraction. ND= not determined. NS= not significant compared to control.

Treatment	Dose (µg/ml)	RAGE expression%		
		PBMCs	Monocytes	Lymphocytes
Glycated serum albumin	100	69.1±8.7	47.2±12.7	41.7±9.8
Glycated serum albumin (100 µg/ml) + R10	10	72.3±11.7	65.2±12.2	55±12.6
	100	86.1±14.3	ND	ND
Glycated serum albumin (100 µg/ml) + whole garlic extract	10	57.4±11.8	42.8±9.5	ND
	100	39.3±5.1	31.7±7.3	ND
R10	10	29.9±5.6	19.6±6.5 NS	29±9.5
	100	39.4±10.3	26±8.7	ND
whole garlic extract	10	19±5.3 NS	16.5±4.2 NS	19.6±6.7 NS
	100	10.6±4.1	15.7±2.6 NS	18.8±5.1 NS
Control		21.2 ±5.9	18.3±5.8	21.6±4.6

albumin were significantly lower as compared to monocytes incubated in the presence of 10 µg dose whole garlic extract plus glycated serum albumin ($P<0.01$).

Although, the whole garlic extract significantly reduced the RAGE expression on PBMCs, it has no significant influence on the RAGE expression by lymphocytes and monocytes. Moreover, in the presence of glycated serum albumin, the RAGE expression on the monocytes and lymphocytes was significantly higher as compared to control cultures ($P<0.0001$ and $P<0.0001$, respectively) but its expression was still lesser than those by PBMCs ($P<0.0001$, $P<0.00001$, respectively). These data confirm that interaction of lymphocytes and monocytes are necessary to mount an immune response to AGEs at least via RAGE.

Table 2: Evaluation of TNFα and IL-1β secretion by PBMCs, monocytes and lymphocytes of diabetic patients by ELISA method before and after the effect of glycated serum albumin, whole garlic extract and its R10 fraction. ND= not determined. *NS*= not significant compared to control.

Treatment	Dose (µg/ml)	TNF-α (pg/ml)			IL-1β (pg/ml)		
		PBMCs	Monocytes	Lymphocytes	PBMCs	Monocytes	Lymphocytes
Glycated serum albumin	100	181±36	105±50 NS	73±33 NS	226±126	166±73	83±45
Glycated serum albumin(100µg/ml) + R10	10	191±68	97±50 NS	84±41 NS	283±117	ND	ND
	100	291±149	ND	ND	345±105	225±94	ND
Glycated serum albumin (100µg/ml) + whole garlic extract	10	129±49	84±37	ND	127±39	92±42	ND
	100	64±27	53±31	ND	75±36	50±25	35±21
R10	10	194±83	106±60	80±47	164±44	ND	ND
	100	215±94	123±58	ND	233±83	ND	ND
whole garlic extract	10	48±12	39±24	49±22	56±20NS	30±18	43±23
	100	29±16	31±23	40±25	53±24 NS	19±12	23±11
Control		84±20	94±39	69±27	55±32	59±24	52±27

The effects of whole garlic extract on inflammatory cytokine secretion in PBMCs, monocytes and lymphocytes. As shown in table 2, whole garlic extract significantly declined TNF-α secretion by PBMCs in a dose-dependent manner. The mean levels of the TNF-α secretion by PBMCs incubated in the presence of 100 µg/ml whole garlic extract were significantly (P<0.001) lower as compared with PBMCs incubated in the presence of 10 µg/ml whole garlic extract. Although the mean levels of the TNF-α secretion by PBMCs in the presence of glycated serum albumin and whole garlic extract (10 or 100 µg/ml) plus glycated serum albumin, were significantly higher as compared with PBMCs from control culture (P<0.001, P<0.01 and P<0.02, respectively), the mean levels of the TNF-α secretion by PBMCs incubated in the presence of 100 µg/ml whole garlic extract plus glycated serum albumin were significantly (P<0.001) lower as compared to PBMCs incubated in the presence of 10 µg/ml whole garlic extract plus glycated serum albumin.

The mean levels of the TNF-α secretion by monocytes in the presence of 10 and 100 µg/ml whole garlic extract were significantly lower as compared with monocytes from control culture (P<0.001, P<0.001, respectively). The mean levels of the TNF-α secretion by monocytes incubated in the presence of 100 µg/ml whole garlic extract plus glycated serum albumin were significantly (P<0.02) lower as compared by monocytes incubated in the presence of 10 µg/ml whole garlic extract plus glycated serum albumin.

The mean levels of the TNF-α secretion by lymphocytes in the presence of 10 and 100 µg/ml of whole garlic extract were significantly lower as compared with lymphocytes from control culture (P<0.05 and P<0.001, respectively).

Glycated serum albumin upraised the TNF-α secretion by PBMCs significantly, but we found no increase in TNF-α secretion by monocytes and lymphocytes separately.

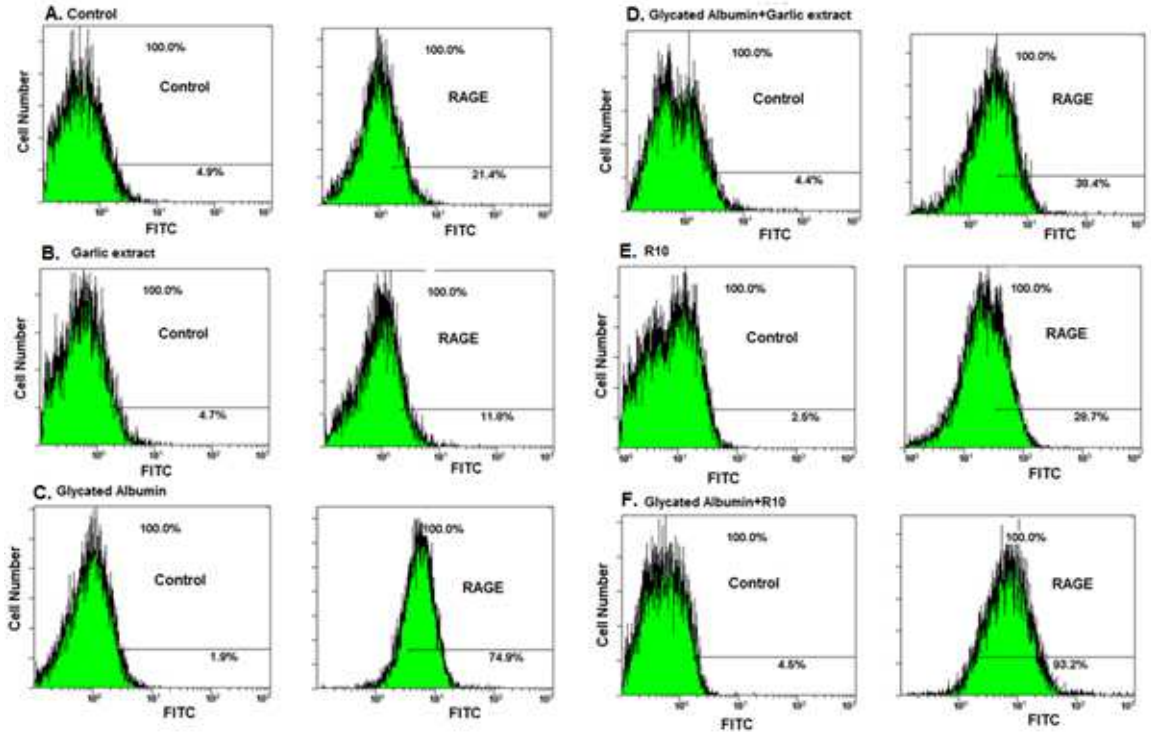

Figure 1. Whole garlic extract down-regulated but R10 fraction and glycated serum albumin up-regulated the RAGE expression on PBMCs. FITC-conjugated mouse anti-human RAGE was used to detect amount of RAGE protein expression on PBMCs before (A) or after the effect of whole garlic extract alone (B) or plus glycated serum albumin (D), glycated serum albumin (C), R10 fraction alone (E) or plus glycated serum albumin (F) on PBMCs by flowcytometry. FITC conjugated mouse IgG was used as control.

Also, whole garlic extract (10 and 100 µg/ml) could not augment IL-1β by PBMCs compared to control, while it increased IL-1β secretion t by monocytes (P<0.001 and P<0.0001, respectively).

No significant difference was observed between lymphocyte cultures of control and whole garlic extract (at dose of 10 µg/ml) regarding the secretion of IL-1β. However, whole garlic extract (at dose of 100 µg/ml) significantly increased the secretion of IL-1β by lymphocytes as compared to control culture (P<0.001). Moreover, although the mean levels of the IL-1β secretion by PBMCs in the presence of glycated serum albumin and whole garlic extract (10 or 100 µg/ml) plus glycated serum albumin, were significantly higher as compared with PBMCs from control culture (P<0.001, P<0.001 and P<0.04, respectively), the mean levels of the IL-1β secretion by PBMCs incubated in the presence of 100 µg/ml whole garlic extract plus glycated serum albumin were significantly (P<0.001) lower as compared to PBMCs incubated in the presence of 10 µg/ml whole garlic extract plus glycated serum albumin.

The mean levels of the IL-1β secretion by monocytes incubated in the presence of 100 µg/ml whole garlic extract plus glycated serum albumin were significantly (P<0.002) lower as compared to monocytes incubated in the presence of 10 µg/ml whole garlic extract plus glycated serum albumin. The glycated serum albumin significantly increased the IL-1β secretion by PBMCs, monocytes and lymphocytes, while the amount of IL-1β secreted by monocytes and lymphocytes was lower than PBMCs.

The mean levels of the IL-1β secretion by lymphocytes in the presence of the 100 µg/ml of whole garlic extract were significantly (P<0.004) lower as compared with lymphocytes in the presence of 10 µg/ml of the whole garlic extract.

Also, the glycated serum albumin augmented IL-1β secretion by PBMCs, monocytes and lymphocytes compared to control, but we found no increase in TNF-α secretion by lymphocytes.

The effects of R10 fraction on RAGE expression in PBMCs, monocytes and lymphocytes. As shown in table1 and fig. 1, in contrast to whole garlic extract, R10 fraction alone or in combination to glycated serum albumin, augmented the RAGE expression by PBMCs in a dose-dependent manner. The mean levels of the RAGE expression by PBMCs in the presence of R10 fraction (10 or 100 µg/ml), R10 fraction (10 or 100 µg/ml) plus glycated serum albumin, were significantly higher as compared with PBMCs from control culture (P<0.005, P<0.001, P<0.001, P<0.001, respectively). The mean levels of the RAGE expression by PBMCs incubated in the presence of 100 µg/ml R10 fraction were significantly (P<0.001) higher as compared to PBMCs incubated in the presence of 10 µg/ml R10 fraction.

The mean levels of the RAGE expression by monocytes in the presence of 10 µg/ml R10 fraction plus glycated serum albumin and 100 µg/ml R10 fraction were significantly higher as compared with monocytes from control culture (P<0.001 and P<0.05, respectively). The mean levels of the RAGE expression by monocytes incubated in the presence of 100 µg dose R10 fraction were significantly (P<0.03) higher as compared to monocytes incubated in the presence of 10 µg/ml R10 fraction.

The mean levels of the RAGE expression by monocytes incubated in the presence of the whole garlic extract (100µg dose) was significantly (P<0.05) lower as compared to monocytes incubated in the presence of the 100µg dose of R10 fraction. We found a significant increase in RAGE expression on PBMCs and lymphocytes treated with 10 µg/ml R10 in comparison with controls, with any significant effect on monocytes. The mean levels of the RAGE expression by lymphocytes in the presence of glycated serum albumin and 10 µg/ml R10 fraction plus glycated serum albumin were significantly higher as compared with lymphocytes from control culture (P<0.001 and P<0.001, respectively).

Although R10 and R10 plus glycated serum albumin significantly increased the RAGE expression on monocytes and lymphocytes compared to control, the RAGE expression was still less in comparison with its expression on PBMCs. Again these data remind that the interaction of monocytes and lymphocytes is necessary to mount an immune response at least via RAGE.

The Effects of R10 fraction on inflammatory cytokine secretion in PBMCs, monocytes and lymphocytes. As shown in table 2, the mean levels of the TNF-α secretion by PBMCs in the presence of glycated serum albumin, 10 or 100 µg/ml R10 fraction, 10 and 100 µg/ml R10 fraction plus glycated serum albumin,

were significantly higher as compared with PBMCs from control culture (P<0.001, P<0.001, P<0.001, P<0.001 and P<0.001, respectively). The mean levels of the TNF-α secretion from PBMCs incubated in the presence of 100 µg/ml R10 fraction plus glycated serum albumin were significantly (P<0.01) higher as compared to PBMCs incubated in the presence of 10 µg/ml R10 fraction plus glycated serum albumin.

Glycated serum albumin and R10 fraction plus glycated serum albumin had no significant effect on TNF-α secretion by monocytes and lymphocytes compared to control. These data confirm that interaction of monocytes and lymphocytes is necessary to mount an immune response (Table 2).

The mean levels of the IL-1β secretion from PBMCs in the presence of 10 and 100 µg/ml R10 fraction, 10 and 100 µg/ml R10 fraction plus glycated serum albumin, were significantly higher as compared with PBMC from control culture (P<0.001, P<0.001, P<0.001 and P<0.001, respectively). The mean levels of the IL-1β secretion from PBMCs incubated in the presence of 100 µg/ml R10 fraction were significantly (P<0.01) higher as compared to PBMCs incubated in the presence of 10 µg/ml R10 fraction

The mean levels of the IL-1β secretion by monocytes in the presence of glycated serum albumin and R10 fraction (100 µg dose) plus glycated serum albumin were significantly higher as compared with monocytes from control culture (P<0.001 and P<0.001, respectively). The mean levels of the IL-1β secretion by lymphocytes in the presence of glycated serum albumin and R10 fraction (100µg dose) plus glycated serum albumin were significantly higher as compared with lymphocytes from control culture (P<0.03 and P<0.05, respectively).

Discussion

Diabetes is recognized as an inflammatory disease caused by a constellation of diverse risk factors [25]. Although many of the classical inflammatory mediators are involved in diabetes, few, if any, of the features of classic inflammation have been observed associated with metabolically triggered inflammation which sometimes referred to as ”low-grade” or ”chronic’ inflammation”. It has been suggested that a constellation of pathways regulating metabolic and immune functions have important roles in this regard. This might allow nutrients to act through pattern-recognizing receptors such as Toll-like receptors (TLRs) and RAGE, thereby inducing chronic inflammatory responses metabolically or nutritionally [26-29].

RAGE and TLRs play a critical role in the innate immune system as they can recognize and interact with microbial products as well as endogenous molecules released in the context of tissue injury and inflammation (i.e. endogenous patterned ligands) such as AGEs [30-32].

The formation and accumulation of AGEs are characteristic features of tissues in aged individuals or patients with diabetes mellitus. These products have also been strongly implicated in the pathogenesis of diabetic micro- and macrovascular complications. It has been demonstrated that AGEs induce insulin resistance by impairing insulin-induced insulin receptor substrate signaling in skeletal muscle cells [33]. Although AGEs form at a constant and slow rate in the normal body, starting in early embryonic development, and accumulate with time in many tissues and contribute to many pathologies [34], if blood sugar remains elevated for prolonged periods (as occurs in poorly controlled diabetics), this may increase glycation and AGE formation up to four times [35].

It is proposed that RAGE activation is strongly responsible for the pathogenicity associated with AGEs [36, 37]. Aside from AGEs, other ligands including S100-calgranulins, high mobility group box-1 (HMGB1), amphoterin, amyloidal-β and other fibrillar proteins can stimulate RAGE [38]. Expression of RAGE enhances in certain cells during diabetes and inflammation. Studies have shown that AGEs upregulate RAGE mRNA levels in pericytes and microvascular endothelial cells [39]. This upregulation may cause an increase in transduction signals following stimulation by AGEs and this may exacerbate loss of pericytes in diabetic retinopathy. Apoptosis of pericytes usually precedes vascular changes and is a characteristic of early retinopathy [40]. This AGE-induced pericytes death has been associated to signaling through RAGE [41].

Studies have shown that blockade of RAGE causes the decreased progression of diabetic retinopathy, while upregulation of RAGE leads to

proinflammatory responses by retinal Müller glia cells [42].

The above-mentioned reports led us to think about some mechanisms to down-regulate the expression of RAGE on inflammatory leukocytes.

In recent years, the use of garlic has been received much interest due to its beneficial effects in protecting against coronary heart disease [43]. Although based on experimental studies, garlic has been shown to exert antilipidemic, antihypertensive, antineoplastic, antibacterial, antiviral, antifungal, antiparasitic, antidiabetic, and immunostimulant activities, its direct anti-inflammatory effects have been received less attention.

In the present study we investigated the influence of garlic extracts on the RAGE expression and proinflammatory cytokines secretion from PBMCs, monocytes or lymphocytes of type 2 diabetic patients.

This study showed the glycated serum albumin, as the most frequent AGE in diabetic patients, up-regulated secretion of TNF-α and IL-1β from PBMCs but the secretion of the proinflammatory cytokines significantly reduced following treatment of cells with whole garlic extract. These data show that garlic can down-regulate the AGEs- mediated inflammation. Furthermore, increase in the RAGE expression on PBMCs with concomitant augmentation of inflammatory cytokines by glycated serum albumin suggests that AGEs may stimulate inflammatory processes at least in part by RAGE signaling in inflammatory cells. In addition, our data of decreased RAGE expression and inflammatory cytokines following exposure to whole garlic extract indicates that garlic suppresses inflammation at least in part by decrease in RAGE expression in inflammatory leukocytes. Our results are partly in line with Youn HS et al. who demonstrated garlic can modulate inflammatory responses through the suppression of TLR activation [44].

On the other hand, our data showed that RAGE, as an innate immune receptor is expressed on monocytes and lymphocytes. Moreover, the glycated serum albumin enhanced IL-1β secretion from PBMCs, monocytes and lymphocytes compared to control, whereas we found no augmentation of TNF-α secretion by monocytes and lymphocytes separately. Hence, our data can partly reinforce the importance of the cooperation of monocytes and lymphocytes in secretion of proinflammatory cytokines Also whole garlic extract significantly down-regulated IL-1β secretion by monocytes and lymphocytes, without any effect on secretion of IL-1β from PBMCs These data may justify the interaction between monocytes and lymphocytes as an important part of the inflammatory responses of the immune system to AGEs ,that in the current study may be mediated via RAGE.

In agreement with our results, several evidence confirms that garlic in addition to well-known nutritional and antimicrobial effects [45], has immunomodulatory activities [46]. However, some studies showed that garlic components stimulate the proliferation of splenocytes [46, 47] and synthesis of the proinflammatory factors [48, 49]. We would like to stress that the evidences about the pro- and anti-inflammatory effects of garlic on the immune system are controversial. There are several reports about some proinflammatory derivatives of garlic such as lectins [50, 51]. To study this issue, we prepared the R10 fraction of garlic in which most garlic lectins are concentrated based on their molecular weight and studied the effect of R10 extract compared to whole garlic extract on PBMCs. Our study confirmed that in contrast to whole garlic extract, R10 fraction alone or in combination to glycated serum albumin, significantly augmented the proinflammatory cytokines secretion and the RAGE expression on PBMCs in a dose-dependent manner. Hence, it seems that it would be better to deplete R10 fraction from garlic to study its anti-inflammatory effects, although we did not study the effect of R10 depleted garlic Collectively, these results shed a new insight into understanding how garlic modulates the immune responses, however, further studies are necessary to elucidate underlying mechanisms to explain the beneficial effects of garlic on chronic diseases such as diabetes.

Conclusion

Downregulation of RAGE expression was associated with decreased secretion of IL-1β and TNF-α from PBMCs after treatment with whole garlic,

while R10 fraction of garlic significantly augmented RAGE expression and proinflammatory cytokines secretion. These data indicates that modulation of RAGE expression may be one plausible reason for the garlic effects on proinflammatory cytokines secretion.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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