

Original Communication

Induction of Th1-dependent immunity by an orally effective melon superoxide dismutase extract

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ABSTRACT

While integrity is essential to confer a pharmacological effect to active enzymes when administered to the oral route, proteins often show a poor bio-availability because they are rapidly eliminated from the alimentary canal before absorption. In order to avoid this problem we evaluated the propensity of a combination of a melon SOD extract (*Cucumis melo*.L.C) with wheat gliadin (*Triticum vulgare*) to be orally effective compared to the melon SOD extract alone. We have previously demonstrated that the combination is the only one to promote the circulating antioxidants (mainly SOD, Gpx and Catalase) and a protective effect to free radicals induced-apoptosis [1]. In this study, we have shown that this combination also primed and polarized the mouse Th1/Th2 immune response by affecting: (a) the production of IFN- γ and IL-4 cytokines from primed spleen cells after stimulation with Con-A and (b) the isotypes of the antibody response (IgG, IgE and IgA).

INTRODUCTION

During the gastro-intestinal transit, orally ingested antigens encounter the gut-mucosal system that is responsible for contradictory roles in preventing immune responses against orally fed antigens [2] while still retaining the ability to respond to potential enteric pathogens. It has been demonstrated that dietary antigens and antioxidants were able to coach the modulation of the immune balance (Th1/Th2) [3,4]

avoiding the induction of the immune system toward "danger" signals. This systemic hypo-responsiveness to orally administered antigen is termed "oral tolerance" [5,6]. Superoxide dismutase (SOD), as part of the antioxidant system, delimits a ubiquitous metallo-enzyme family involved in the dismutation of superoxide anion into hydrogen peroxide. Previous results have shown that even though different SODs have the same enzymatic activity, their biological effectiveness is not equal facing the recipient background [7,8]. This statement was confirmed in different human clinical trials wherein the purified bovine Cu/Zn-SOD was fully active while the human one was not [9-11]. We assumed that heterologous SODs could act, *in vivo*, by modulating the mucosal immune response. The subsequent polarization of the immune system could be responsible in great part for their anti-inflammatory properties. In the present study we tested an oral composition taking benefit of the bio-adhesive properties of the wheat gliadin to delay the release of a vegetal superoxide dismutase (*Cucumis melo* L.C.) throughout the digestive process [12,13] and thus ensuring its oral bioactivity [14,15]. We demonstrated that this combination, also named Glisodin[®], was able to stimulate the host antioxidant system by inducing a Th1-polarization of the immune response through the expression of IFN γ and an antibody response of IgG isotype.

RESULTS AND DISCUSSION

Supplementation with Glisodin[®] induced the host antioxidant system

The gliadin was chosen for its strong adhesive capacity

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with gastro-intestinal mucosa in order to extend the resident time of the melon SOD extract and therefore increases the time when absorption can occur [12,13,16]. Tacking into account that the gliadin-combination delays the release of the melon SOD through the gastro-intestinal transit [1] and can address the SOD through the intestinal mucosa [17], we expected an increased absorption of the melon SOD extract with subsequent changes in the rate of circulating SOD.

Blood samples were periodically isolated (each 7 days) from six weeks old BALB/c mice (IFFA-CREDO, Orleans, France) that received daily during 28 days a control diet supplemented either with 10IU of free vegetal SOD (G0) or with 1IU of the orally bioactive SOD (G1) Glisodin®. The determination of enzymatic activities were then performed and revealed that more than an uptake of the melon SOD into the circulation, Glisodin® ingestion induced an increase of the resident circulating SOD activity [18] in association with Catalase [19] and Gpx [20] activities (table.1) to maintain the essential oxidant/antioxidant balance [21]. According to the fact that Glisodin® administration induced a dynamic biological process that promotes the circulating antioxidant system we evaluated the priming effect of repeated feeding with this bioactive SOD on the immune system.

Supplementation with Glisodin® primed the Th1 immune response.

Spleen cells were periodically sampled (each 7 days during 28 days) from BALB/c mice selected from the group that received daily 1 unit of the orally bioactive SOD (G1). After 48 hours of incubation with 10 µg/ml

Table 1.
Effect of orally bioactive SOD on circulating antioxidants,

	SOD (UI/gHb)	Gpx (UI/gHb)	Cat (KUI/gHb)
G0	1720 ± 125	800 ± 33	35 ± 5
G1	3250 ± 255	1210 ± 89	95 ± 6

Balb/c mice were daily fed either with G0 (free melon SOD extract) or with G1 (Glisodin®) during 28 days. Blood samples were collected to measure the SOD, Gpx and Catalase activities. Data represent the mean ± SD of ten animals/group from one representative experiment.

of Con-A, stimulated splenocytes (5×10^5 cells/ml) revealed a production of IFN-γ (ELISA kits from Pharmingen, France) that progressively and more substantially increases than the IL-4 one, together with the time-span of the Glisodin diet, (Figure.1). We previously showed that the wheat gliadin alone was able to promote the IgE production by normal human PBMC in presence of IL-4 [23]. Herein, its combination with the melon SOD extract (Glisodin®) was found to increase the IFN-γ production and modulate the IL-4 one in BALB/c mice.

It is known that IFN-γ produced by the Th1 subset have the dual effect of both stimulating Th1 and inhibiting Th2 developments [22] subsequently decreasing the IL-4 levels. This suggested a progressive polarization of the immune system toward the production of Th1-dependent cytokines with a maximal effect observed after 28 days of supplementation. According to this result, if Glisodin® is responsible for skewing BALB/c mice Th1/Th2 response, we were expecting an additional switch over for the isotype of the antibody response.

Total IgA, and IgG were analyzed in the sera by specific ELISA tests using Micro-plates (Maxisorp

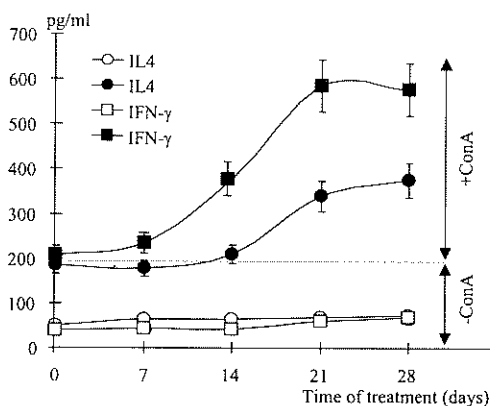


Figure 1: Cytokines production under a supplementation with Glisodin® : Splenocytes were periodically sampled (from day 0 to day 28) from BALB/c mice supplemented daily with 1 mg of (1 unit) Glisodin®. Basal level are considered at J0, each mouse being its own control. After Con-A stimulation the production of IL4 and IFNγ were evaluated and compared. Data represent the mean ± SD of the different groups.

Nunc, Polylabo) coated with primary antibodies, respectively 3 $\mu\text{g/ml}$ of rabbit anti-mouse IgA or 3 $\mu\text{g/ml}$ rabbit anti-mouse IgG (Sigma). Levels of serum IgE were determined with the monoclonal antibodies R35-72 and R35-92 supplied by Pharmingen (Les Ulis, France). As expected, oral administration of Glisodin® elicited increased Th1 response manifested by increased expression of IFN γ and a bulky IgG production after a period of 28 days (Figure.2, $p < 0.01$). Despite the presence of gliadin into the combination the production of IgE remained moderate while the production of IgA remained unchanged strengthening the hypothesis that the Glisodin® product acts as an immuno-modulator.

The instructive role of immunity in the mechanism of action of Glisodin®: hypothesis.

We can assume that the Glisodin®-dependent induction of a Th1-dependent immune response is responsible for the antioxidant mobilization in tested animals (Figure. 3). Indeed, due to the capacity of gliadin to interact with the HLA class II antigens mainly on epithelial cells in the gut and on macrophages. It is expected that the active SOD should be transported through the

intestinal mucosa and delivered in the cytoplasm of antigen presenting cells, APC [24]. The consequence of this activation process (Phase I) is: first, the induction of H $_2$ O $_2$ and nitric oxide production that finally restores the natural antioxidant balance in inducing Gpx and catalase expression and second: the antigen processing and presentation of the resulting SOD to finally activate cytokines expression. Following the phase I, the antigen presenting cells (APC) polarize the adaptative immune system (phase II) and, especially, T cells to produce preferentially IFN- γ and B cells to produce non sensitizing immunoglobulins [25]. Glisodin®-dependent Th1 polarization promotes the natural cellular and tissues antioxidant equilibrium (phase III) through IFN γ [26] and immune [24] dependent mechanisms.

DISCUSSION

Like many proteins, SODs are usually poorly efficient when administered to oral route, because they are rapidly eliminated from the alimentary canal before being absorbed.

Associations of different kinds of SOD with various natural or synthetic polymers were tested in order to protect or at least to delay their degradation in the host [15,27]. In this work, the gliadin was chosen for its adhesive capacity with the gastro-intestinal mucosa, to extend the resident time of the product in the gut, thus increasing the time when absorption of the melon SOD extract can occur [12-14,16]. Another property of gliadin is the activation of the zonulin pathway from intestinal cells leading to tight junction opening [28,29]. The subsequent increase in intestinal permeability may allow the trans-epithelial passage of macromolecules such as the melon SOD [30,31]. In this scheme, a significant dose of active SOD can be transported through the upper-intestinal mucosa and presented to specialized antigen-presenting cells, APC. Results also suggest that after being captured by the APC, the SODs need to express their heterologous origins to be fully effective [32,33]. The subsequent induction of a Th1-dependent immune response then contributes to the recruitment of natural antioxidant defences (SOD, catalase and Gpx) [34] protecting cells to stress and death (by apoptosis). This immune-dependent mechanism of the bioactive melon SOD is mandatory because it maintains or restores the cellular redox balance when impaired and thus reduces the risk of free radicals toxicity [35].

Thus far, SOD were wholly considered as antioxidant enzymes but its now evident that heterologous SOD displayed additional immuno-modulatory properties through their antigenic profile.

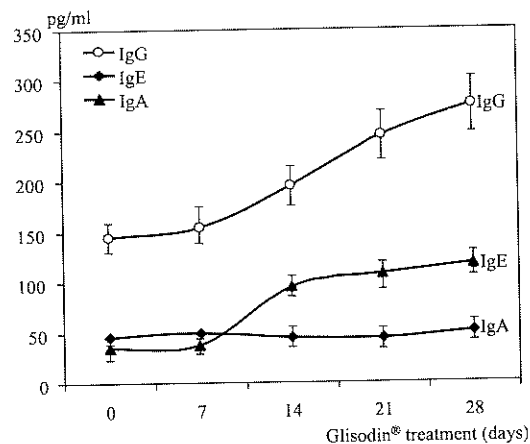


Figure 2: Antibody response under a supplementation with Glisodin® : Blood was periodically sampled (from day 0 to day 28) from BALB/c mice supplemented daily with 1 mg (1 unit) of Glisodin®. The progressive production of each Isotype was assessed by ELISA and Data represent the mean \pm SD of the different groups.

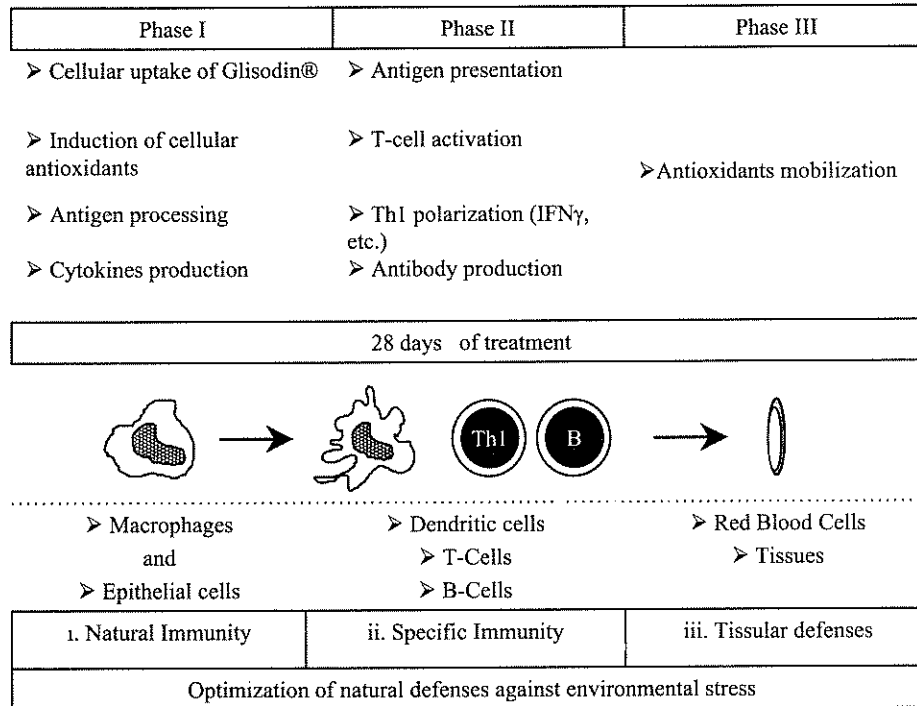


Figure 3

While injections of bovine SOD were largely used in many human trials, this heterologous (antigenic) molecule did not induced dramatic side effects. So it is likely that heterologous SODs (injected or orally bioactive) stimulate the natural mucosal immune system to promote a tolerance response. Based on the results mentioned above, it is reasonable to believe that dietary supplementation of SOD combined with wheat gliadin may influence not only the expression of circulating and tissue SOD activity but also the concomitant expression of other antioxidant enzymes, catalase and GPx .

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