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Original article

Superoxide Dismutase (SOD), a Powerful Antioxidant, is now available Orally

F. Joanny Menvielle-Bourg

Doctor in Pharmaceutical Sciences, Fabienne Joanny, Consulting & Licensing, Paris, France

Correspondence: e-mail: fabienne.joanny@joanny-consulting.com

Abstract: Oxidative stress, the natural consequence of the oxygen metabolism, is normally controlled by antioxidant endogenous defense systems. When these prove to be insufficient, cellular lesions develop that result in ageing but also in some pathological processes. The powerful natural antioxidant enzyme superoxide dismutase (SOD) acts at the very source of the chain reaction resulting in reactive types of oxygen and therefore constitutes the first and one of the main links of the defense process against free radicals. Unfortunately, due to the fragility of its molecular structure, non-protected SOD is inactivated in the digestive tract. Thanks to a coupling process with gliadin, a protein extracted from wheat, a SOD of vegetable origin (melon extract rich in SOD) is now available orally. Several *in vivo* studies on animals as well as a clinical trial using healthy volonteers confirmed the preservation of the antioxidant activity of the SOD enzyme after oral administration; an action moreover combined with anti-inflammatory and immunomodulatory properties.

Keywords: Superoxide dismutase (SOD) – Gliadin – Vegetable origin – Oral bioactivity – Antioxidant action – Anti-inflammatory and immunomodulatory properties – Free radicals – Degenerative and diseases and ageing – Clinical trial – *In vivo* studies

La superoxyde dismutase, puissant antioxydant naturel, désormais disponible par voie orale

Résumé : Le stress oxydatif, conséquence naturelle du métabolisme de l'oxygène, est normalement contrôlé par des systèmes de défense antioxydante endogènes. Lorsque ceux-ci s'avèrent insuffisants, il en résulte des lésions cellulaires impliquées dans le vieillissement mais également dans certains processus pathologiques. Puissante enzyme antioxydante naturelle, la SOD agit à la source même de la réaction en chaîne induite par les espèces réactives de l'oxygène et constitue donc le premier et l'un des principaux maillons du processus de défense contre les radicaux libres. Malheureusement, en raison de la fragilité de sa structure moléculaire, la SOD non protégée est inactivée dans le tube digestif. Grâce à un procédé de couplage à la gliadine, protéine extraite du blé, on dispose désormais d'une SOD d'origine végétale (extrait de melon riche en SOD) bioactive par voie orale. Plusieurs études *in vivo* chez l'animal ainsi qu'une étude clinique chez des volontaires sains ont effectivement confirmé le maintien de l'activité antioxydante de l'enzyme SOD après absorption par voie orale, action par ailleurs combinée à des propriétés anti-inflammatoires et immunomodulatrices.

Mots clés : Superoxyde dismutase (SOD) – Gliadine – Origine végétale – Bioactivité par voie orale – Action antioxydante – Propriétés anti-inflammatoires et immunomodulatrices – Radicaux libres – Maladies dégénératives et vieillissement – Étude clinique – Études *in vivo*

Free Radicals, a Consequence of Oxygen Metabolism

Oxygen is absolutely necessary for the life processes, in particular cell respiration. However, the metabolism of oxygen may generate reactive elements called free radicals, in particular the superoxide ion (O_2^-) and the hydroxyl ion (OH^-). These chemically unstable compounds carry free electrons that react with other molecules, in turn destabilizing them and thereby inducing a chain reaction. In particular, free radicals damage DNA, essential cellular proteins and membrane lipids (lipid peroxidation), which may lead to cell death.

In so-called "physiological conditions" there is a balance between the production of free radicals and antioxidant endogenous defense mechanisms. These mechanisms mainly involve specific enzymes (superoxide dismutase or SOD, catalase, gluthation peroxidase or Gpx) as well radical scavengers that trap free radicals (antioxidant vitamins A, C, E), thiols and ß-carotene) [4].

However, certain conditions accompany the increased production of unstable oxygen derivatives: metabolism of sugars related to physical stress, lipid metabolism immune response in particular toward microbial infections, exposure to radiation, pollution, smoking… Moreover, epidemiology studies indicate that the level of the antioxidant defenses

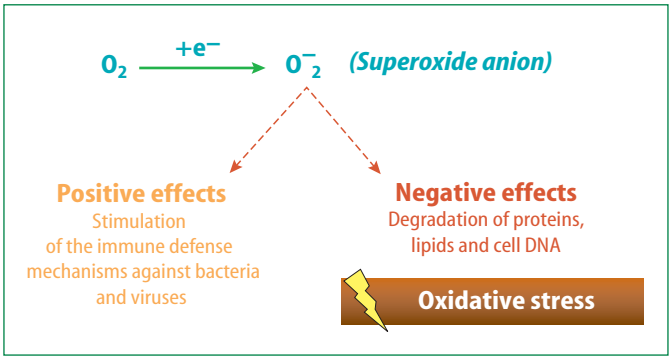

Fig. 1. Effects of oxidative stress

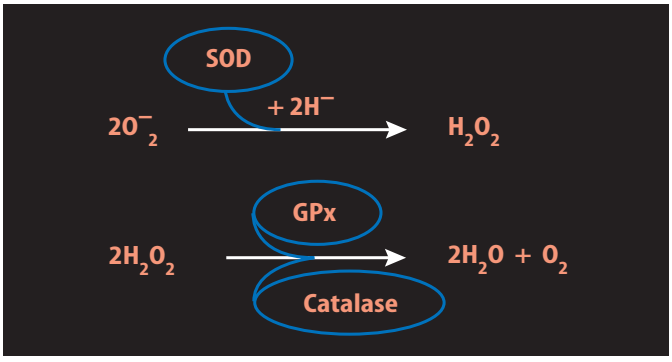

Fig. 2. Role of antioxidant enzymes in the inactivation process of superoxide ion

decrease with age. When the antioxidant systems of defense are overloaded, oxidative stress (free radicals in excess) may occur. This may eventually contribute to the development of inflammatory or degenerative diseases (Fig. 1).

The important role of oxidative stress is well known in a great many diseases: neurodegenerative diseases (Alzheimer's disease, Parkinson's disease), atherosclerosis, rheumatoid arthritis, Crohn's disease and even certain cancers. Free radicals are also known to contribute to the aging process. For this reason, we are currently witnessing the development of a great many antioxidant products (functional food and drugs). However, their bioactivity with oral administration is often low, thereby limiting their efficacy. In addition, the products available on the market are made to correct a possible deficiency and do not specifically stimulate the antioxidant endogenous defenses.

Superoxide Dismutase (SOD): An Essential Link in the Fight against Free Radicals

The determinant role of superoxide dismutase (SOD) in the antioxidant defense systems has been known since 1968. It is well known that superoxide ion (O_2^-) is the starting point in the chain production of free radicals. At this early stage, superoxide dismutase inactivates the superoxide ion by transforming it into hydrogen peroxide (H_2O_2). The latter is then quickly catabolised by catalase and peroxidases into dioxygen (O_2) and water (H_2O) (Fig. 2). Different studies have confirmed that the production of H_2O_2 under the action of SOD is the triggering factor in the natural antioxidant defense mechanisms. SOD therefore seems to be the key enzyme in the natural defense against free radicals (Fig. 3).

GliSODin®: the First Bio-active SOD Available Orally

SODs are protein enzymes and their function specifically depends on their quaternary structure. All changes in the environment may, to a greater or lesser extent and more or less irreversibly, modify this structure and therefore the functionality of the SOD. In particular, during gastro-intestinal passage, the quaternary structure is modified and the enzyme is inactivated. This is why it is difficult to produce a SOD-rich food supplement that remains active when

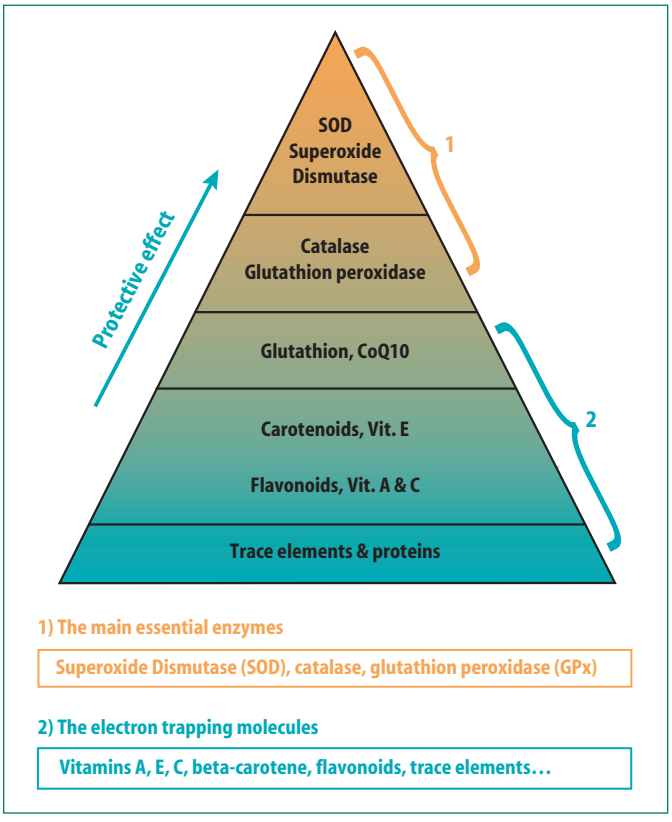

Fig. 3. SOD among the other electron trappers: vitamins A, E, C, beta-carotene, flavonoids, trace elements…

taken orally [1]. Therefore, to guarantee its efficacy, a SOD of exogenous origin has to be bioavailable, active in the body and protected during its digestive passage.

Glisodin® is an original vegetable formula made from a SOD-rich melon extract (*Cucumis melo* LC), coupled with a Gliadin molecule, a protein extracted from wheat (Glisodin®) (Fig. 4).

Gliadin is a vegetable prolamine (biopolymer) that retains the active ingredient and delays its release in the small intestine. It is also bio-adhesive and in particular adheres to the wall of the small intestine (Fig. 5). It progressively releases the SOD, counters its intestinal inactivation

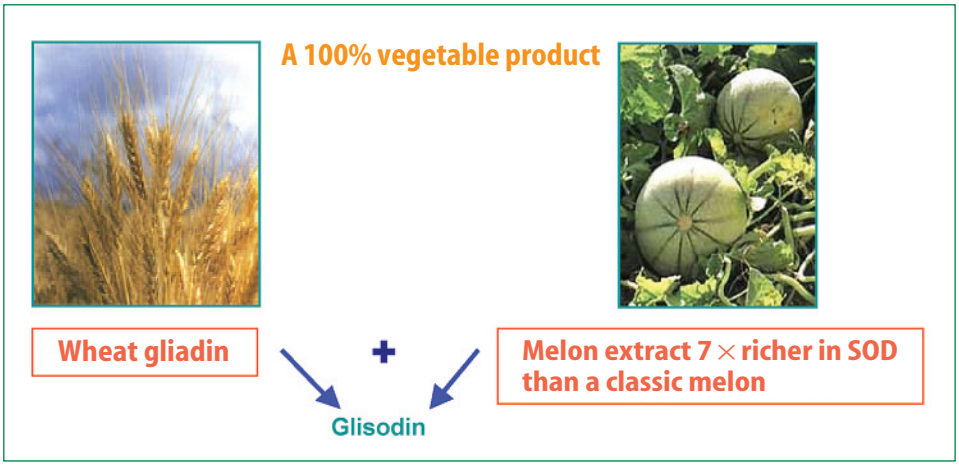

Fig. 4. Glisodin®, an original vegetable formula

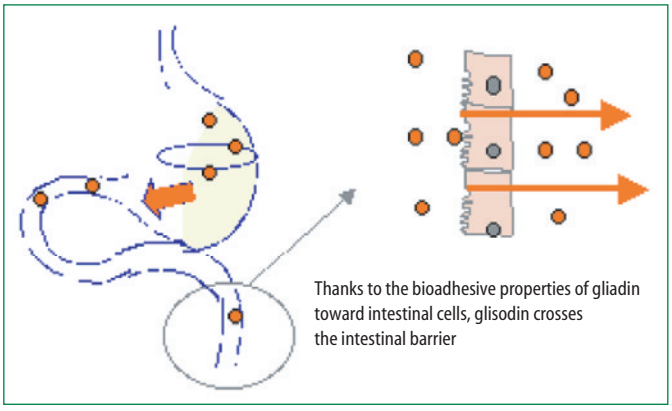

Fig. 5. Diagram of intestinal passage and absorption of SOD coupled with gliadin

and eases its passage through the mucosa towards the blood circulation. Therefore, Glisodin® is the first active SOD orally available.

Scientifically Verified Efficacy

In vitro *studies*

A great many *in vitro* studies have been carried out on SOD, largely demonstrating its antioxidant action. Regarding the SOD-rich melon extract, this property has been confirmed in a study carried out on a murin macrophage model in culture, activated by interferon-gamma (INF-gamma) then put in the presence of IgGl/anti-IgG1 immunocomplexes (IgG1IC complexes) [5]. The melon extract has been shown to inhibit the production of superoxide and peroxynitrite ion by thereby activated cells in a dose-dependant manner.

Animal studies

Considering the specific properties claimed, it was important to check, by *in vivo* studies, whether Glisodin® protects the SOD molecule and thereby preserves the enzyme function and biological properties of SOD after oral administration.

Antioxidant properties

An *in vivo* study was carried out on Balb/c mice receiving an oral supplement by a standardised SOD melon extract, administered alone or combined with gliadin (Glisodin®) over a period of twenty-eight days [4]. Several bio-markers of oxidative stress were analysed to objectify the effects in both groups:

- enzymatic activity of erythrocyte SOD, Gpx and catalase;
- hemolysis provoked by a free radical inducer (AAPH);
- peroxynitrite-induced apoptosis in hepatic cells with or without Sin-1;
- mitochondrial depolarization in hepatic cells.

As expected, no significant change in the parameters analyzed was noted with the non protected SOD. However, a significant increase in the activity of circulating antioxidant enzymes was noted in the animals receiving Glisodin®. This was correlated with a higher resistance of erythrocytes with respect to the hemolysis induced by oxidative stress. In the presence of Sin-1, chemical precursor of peroxynitrites, hepatic mitochondria underwent membrane depolarization, an early biological phenomenon in apoptosis. Hepatic cells isolated from animals receiving Glisodin® presented a delayed depolarization response and an increase in the resistance to apoptosis induced by oxidative stress. Therefore, Glisodin® supplementation seems to increase the antioxidant status of cells and protects them against the lethal risk resulting from oxidative stress.

Anti-inflammatory properties

Another study, carried out in C57BL/6 mice analyzed the anti-inflammatory properties of Glisodin® after oral administration for twenty-eight days [5]. Gliadin alone, melon extract alone and a placebo were also tested. After the supplementation period, peritoneal macrophages were activated *in situ* by INF-gamma via the IP route and then collected twenty-four hours later. Their ability to produce free radicals and cytokines (*tumour necrosis factor* or TNF-alpha and interleukine-10 or IL-10) was measured after incubation in the presence of IgG1IC immunocomplexes. Only Glisodin® was found to protect the cells from the pro-

inflammatory action of INF-gamma. This protection resulted in a significant increase in the production of IL-10 and a significant reduction in the production of TNF-alpha after activation of macrophages by immunocomplexes. Although involvement of other components of the melon extract cannot be totally excluded, the antioxidant effect of SOD very probably plays a basic role in the anti-inflammatory properties observed.

Immunomodulatory properties

The same treatment protocol (twenty-eight days, oral administration) was used in C57BL/6 mice in order to assess the immunostimulant potential of Glisodin® (1 IU) [3]. Blood samples confirmed that Glisodin® increases the activities of the circulating SOD enzymes, catalase and Gpx. In addition, spleen cells were collected each week during the supplementation period. Glisodin® was found to increase specifically the production of type 1 helper T lymphocytes (Th1) as well as the expression of INF-gamma and IL-4 and to stimulate the immunoglobulin G response. However, the production of IgE (allergic) remained marginal and the production of IgA did not change, thereby reinforcing the hypothesis of the immunomodulatory action of Glisodin®. This action might result from the activation of antigen-presenting cells (APC) by the gliadin-SOD combination. This activation would induce the release of nitric oxide (NO) and H_2O_2, in turn inducing the activation of catalase and Gpx, followed by the activation of the expression of the INF-gamma and IL-4 cytokines. The immune response should be then polarised by the activated APC towards a Th1 response.

Clinical study in man

A randomised, double-blind, controlled clinical trial *versus* placebo evaluated the protective power of Glisodin® from cellular oxidative stress induced by hyperbaric oxygen (HBO) [2]. Twenty healthy volunteers were exposed to pure oxygen (pressure of 2.5 absolute atmospheres) for sixty minutes. The rupture of DNA strands was detected using the comet assay, determining the tail moment (produced by the length of the comet by the percentage of DNA in the distal part). Blood concentrations in reduced (GSSG) and oxidised (GSH) glutathion and in F2-isoprostanes, SOD, Gpx and catalase activities and finally malondialdehyde concentration (MDA) in erythrocytes were also analysed.

After exposure to HBO, a considerable reduction was noted in the tail moment and the isoprostane concentration in the Glysodin® group ($p=0.03$ and $p=0.049$ respectively). Neither the activity of the SOD and catalase, nor the GSH and GSSG concentrations were considerably affected by the supplementation or by exposure to HBO. However, the activity of circulating Gpx, that already tended to be lower in the Glisodin® group before exposure to HBO ($p=0.076$) appeared considerably lower thereafter ($p=0.045$). Therefore, after the oral administration in healthy volunteers, Glisodin® manifests an incontestable biological activity resulting in a protective effect from DNA damage induced by hyperbaric oxygen, thereby confirming the powerful antioxidant properties of SOD.

Conclusion

Glisodin® is the first SOD effective with oral administration. Contrary to other products available on the market, that act more downstream and are subject to a saturation phenomenon due to their essentially antiradicular properties, SOD induces the activation of the endogenous system of antioxidant defenses. As a result, Glisodin® is a unique food supplement, especially appropriate in the fight against free radicals overloading, in particular when the body's own natural defenses are weakened: elderly subjects (reinforcement of the general state), exposure to sun (prevention of allergies), smoking, stress, intense physical exercise… It prevents certain chronic disorders involving oxidative stress or slows down their evolution, thereby improving the patient's conditions of life.

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