THE ORALLY EFFECTIVE MIXTURE OF SOD AND GLIADIN *GliSODin®* PROTECTS AGAINST OXIDATIVE DNA DAMAGE

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Background:

Generation of oxygen-derived radicals has been demonstrated to be the major mechanism of ischemia-reperfusion induced damage [1]. Reactive oxygen species interact with DNA leading to structural alteration [2]. Recently, we showed that *GliSODin®*, an orally effective mixture of SOD and wheat gliadin, protected against hyperbaric oxygen (HBO)-induced DNA damage assessed by single cell gel electrophoresis (comet assay) [4]. Since DNA damage was less marked than in previous studies [3], we investigated whether *GliSODin®* is also protective when during more severe oxidative stress.

Materials and Methods:

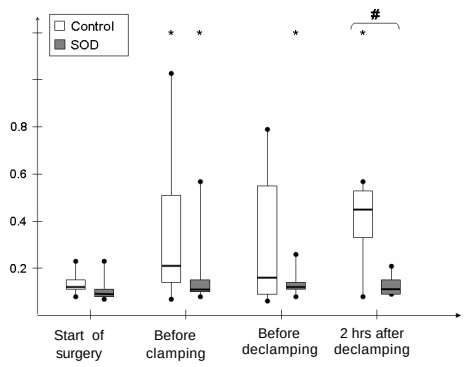
After 2 weeks of feeding once a day with 1250 IU of *GliSODin®* (n = 9) or vehicle (n = 5) blood samples were taken from swine (body weight 50 (47-53) kg). DNA damage (tail moment in the alkaline version of the comet assay) was evaluated in isolated lymphocytes (Ficoll gradient) before and after HBO exposure (2 hrs at 4 bar O_2) (*GliSODin®* n=9, Vehicle n=5). Comet assay was performed as well in whole blood samples taken at different time points (before and after 30 minutes thoracic aortic cross-clamping as well as 2 and 4 hours after declamping) during thoracic aortic surgery (*GliSODin®* n=8, Vehicle n =7). Plasma 8-isoprostane (8-epi Prostaglandin F_2) concentrations as a direct marker of lipid peroxidation were determined using an enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI) in portal (PV) and hepatic venous (HV) blood before and after aortic cross clamping. Antioxidant enzyme SOD (RANSOD kit, Randox Laboratories Ltd, U.K.) and catalase activity (assayed by a method in which the disappearance of peroxide is followed spectrophotometrically) were assessed on whole blood samples taken during aortic cross clamping and on lymphocytes isolated before and after HBO exposure. Data are median (range). After exclusion of normal distribution data using a Kolmogorov-Smirnov-test, time dependent differences within groups during aortic surgery were analyzed with a Friedman repeated measures ANOVA and, if appropriate, by a Dunn's test. Differences before and after HBO exposure were analyzed with a Wilcoxon signed rank test. Intergroup differences were analyzed with Mann-Whitney rank sum test.

Results

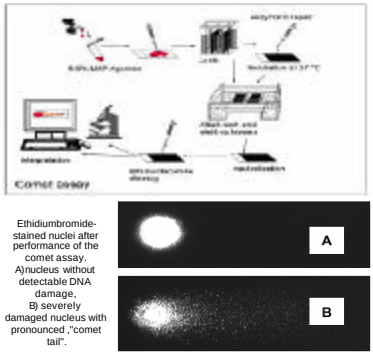
There was no difference in DNA damage before exposure to HBO (p= 0.255 *GliSODin®* vs. Vehicle). *GliSODin®* bounded (tail moment from 0.08 (0.06-0.12) to 0.11 (0.07-0.23), p=0.020) the otherwise marked increase (Vehicle: tail moment from 0.11 (0.09-0.13) to 0.43 (0.40-0.73), p=0.063) in DNA strand breaks after HBO exposure (p=0.005 *GliSODin®* vs. Vehicle after HBO).

GliSODin® also reduced oxidative DNA damage related to surgical stress and ischemia-reperfusion after aortic clamping (tail moment from 0.09 (0.08-0.11) to 0.12 (0.11-0.14), p= 0.023) in comparison to Vehicle (tail moment from 0.12 (0.11-0.15) to 0.48 (0.35-0.60). p=0.031) resulting in a statistically significant intergroup difference 2 hrs after declamping (p= 0.021, *GliSODin®* vs. Vehicle). While there was no intergroup difference in the baseline values of isoprostane levels (p=0.391), these values significantly increased both in hepatic venous (p=0.003) and portal venous samples (0.006). No such effect was observed in *GliSODin®* group. Neither SOD nor catalase activities were significantly affected by *GliSODin®* ingestion (baseline values *GliSODin®* vs. Vehicle, p= 0.289) or by stress related to surgery and aortic cross clamping (*GliSODin®* vs. Vehicle p= 0.312).

COMET ASSAY CLAMPING

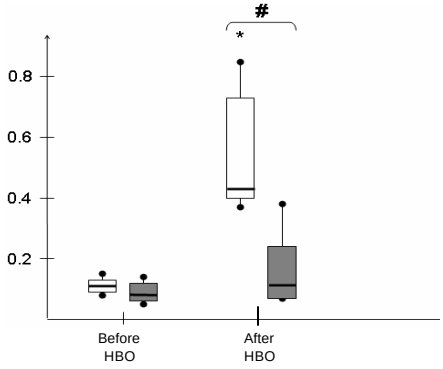


* p<0.05 vs baseline. # p<0.05 vs Vehicle



Ethidiumbromide-stained nuclei after performance of the comet assay. A) nucleus without detectable DNA damage, B) severely damaged nucleus with pronounced „comet tail".

COMET ASSAY LYMPHOCYTES IN HBO



* p<0.05 vs baseline. # p<0.05 vs Vehicle

8-ISOPROSTANE pg / g protein		before clamping	2 hrs after declamping	4 hrs after declamping
HEPATIC VEIN	*GliSODin®*	**1945** (1289-2097)	**1797** (1540-2203)	**1716** (1356-1993)
	Vehicle	**1762** (1528-1898)	**2188*** (1837-2645)	**1842** (1655-1999)
PORTAL VEIN	*GliSODin®*	**2309** (1700-2419)	**2339** (1990-2637)	**1958** (1428-2604)
	Vehicle	1853 (1568-2028)	**2264*** (1946-2691)	**2091** (2012-2148)

		before clamping	2 hrs after declamping	4 hrs after declamping
SOD [U/gHb]	*GliSODin®*	**1.6** (1.3-1.7)	**1.6** (1.4-1.7)	**1.6** (1.3-1.8)
	Vehicle	**1.4** (1.3-1.5)	**1.5** (1.4-1.7)	**1.4** (1.3-1.6)
Catalase [kU/gHb]	*GliSODin®*	**80** (77-88)	83 (79-92)	**81** (77-87)
	Vehicle	**82** (74-86)	**80** (71-87)	**86** (72-87)

		SOD Mn [U/gHb]	SOD CuZn [U/gHb]	SODt [U/gHb]
Before HBO	*GliSODin®*	**1.2** (0.7-1.3)	**2.2** (1.9-2.8)	**3.4** (3.2-3.8)
	Vehicle	**1.3** (1.0-1.4)	**3.1** (2.5-3.8)	**4.2** (3.8-4.8)
After HBO	*GliSODin®*	**1.2** (1-1.3)	**2.7** (2.5-3.2)	**3.9** (3.5-4.3)
	Vehicle	**1.3** (1.1-1.5)	**2.8** (2.3-3.5)	**4.2** (3.8-4.6)

*: before clamping vs 2 hrs after declamping

Conclusions:

Pretreatment with the new nutritional formula of SOD-wheat gliadin (*GliSODin®*) allows to prevent oxidative DNA damage related to HBO treatment or ischemia-reperfusion injury.

The unaltered SOD activities after oral SOD ingestion are probably due to relatively low SOD supplementation when compared to total blood SOD pool [3,5]. The effect of SOD, thus, most likely results from an immune response and through a nitric oxide dependent mechanism [5].

References:

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