Nitric oxide metabolism and DNA breakage in autoimmune diseases

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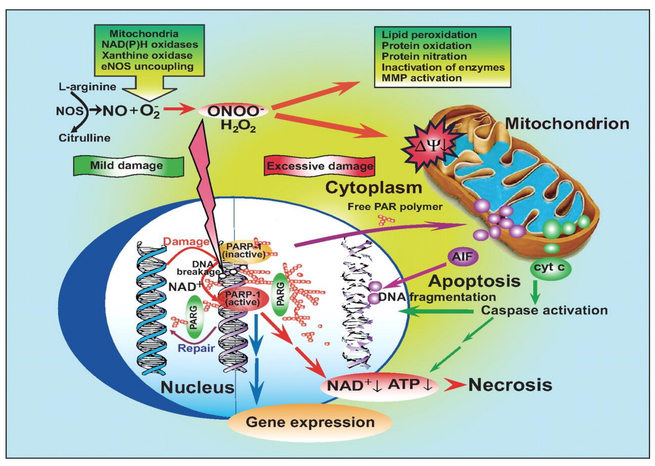
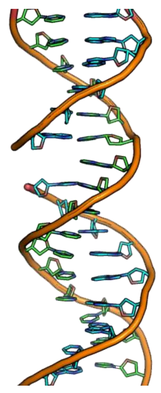
Background

Oxidative stress is involved in the pathogenesis of multiple sclerosis and diabetes mellitus, and might lead to DNA damage. One of the sourses of oxidative stress in chronic inflammatory diseases might be overproduction of nitric oxide and eNOS uncoupling.



Pacher P., Beckman J. S., Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev,* 2007, **87**(1): 315-424.

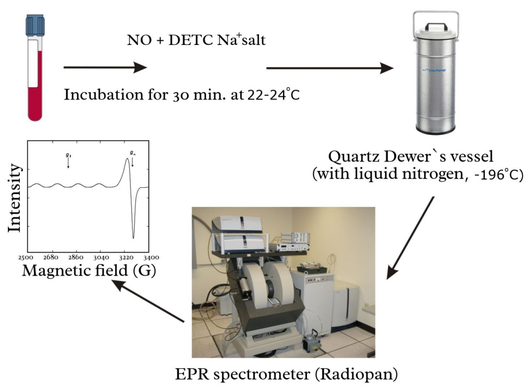


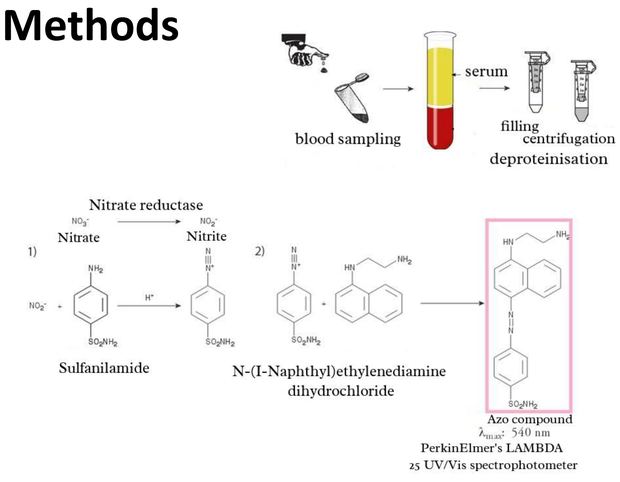
3D model of the single-stranded DNA break

Aim

To characterize NO production, NO_2/NO_3 concentration and single-strand DNA breaks in the biological fluids of patients with type 1 diabetes, multiple sclerosis and healthy subjects.

Methods





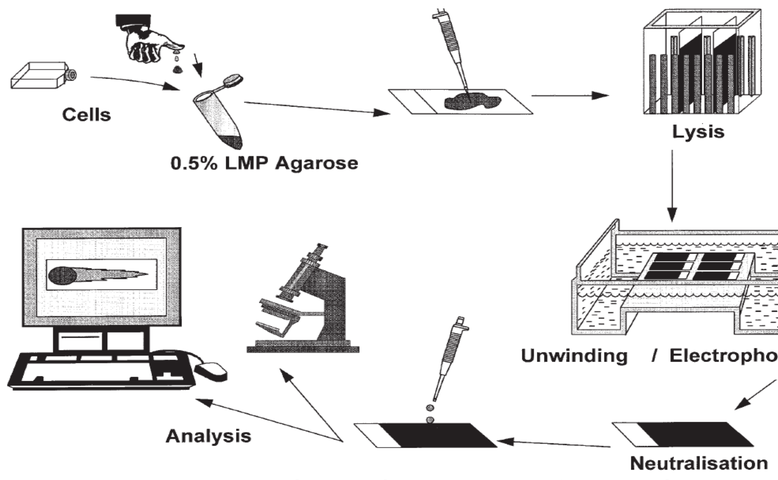
EPR

Wholeblood: measures how much NO blood cells produce

Griess reaction

Serum: estimate of NO production by endothelium and other organs

Urine: estimate of NO production by kidney+spillover from serum



Comet assay: Daryl S. Henderson DNA repair protocols, 2006

Subjects

Comet assay

28 MS patients: 10 males, 18 females, age 39.5 ± 1.9 years
15 healthy subjects: 2 males, 13 females, age 34.7 ± 3.3 years
88 T1D patients: 41 males, 47 females, age 36,82 ± 12,33 years
44 healthy subjects: 19 male, 25 female, age 32,14 ± 14,16 years

EPR (NO production)

22 MS patients: 8 males, 14 females, age 39.0 ± 1.9 years;
22 healthy subjects: 7 males, 15 females, age 30.6 ± 3.2 years
203 T1D patients: 100 male, 103 female, average age 36(24-47)
69 healthy subjects: 26 male, 43 female, average age 23(21-26)

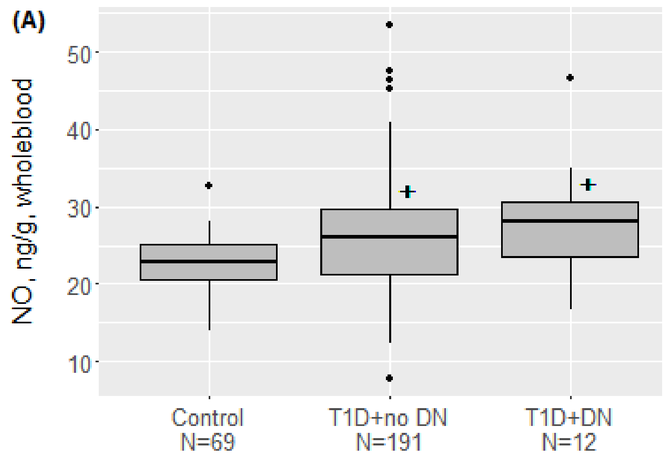
NO2/NO3 (Griess reaction)

271 T1D patients: 124 male, 147 female, age 36(24-47**);**
39 healthy subjects: 19 males, 20 females, age 24(22-27)

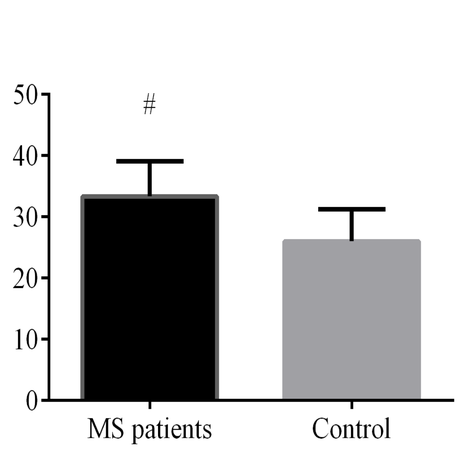
Results

NO production in wholeblood

NO was higher in patients with diabetes, compared to healthy subjects ($p<0.001$). Diabetic nephropathy was associated with further increase of NO concentration



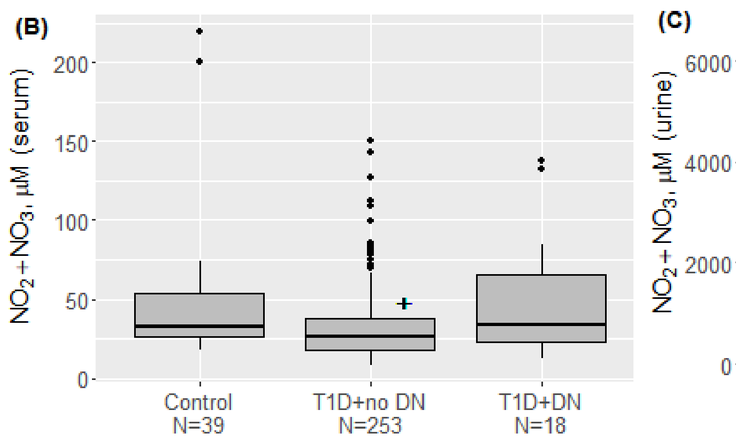
NO production in blood of healthy subjects, patients with uncomplicated type 1 diabetes and diabetic nephropathy (DN) (ng/g tissue). + - p< 0.05 vs control.



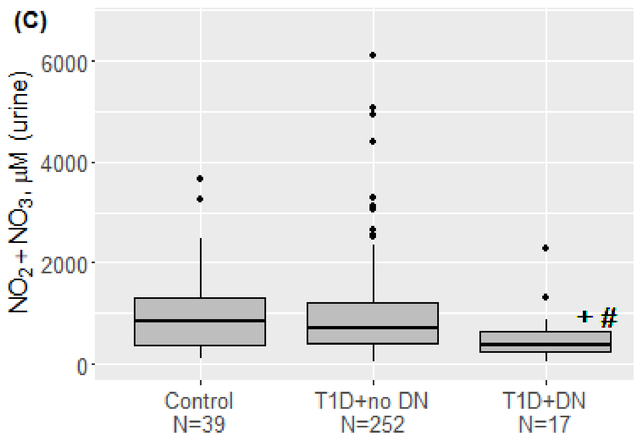
NO production in blood of MS patients and healthy controls (ng/g tissue). # - p< 0.05 vs control.

NO2/NO3 concentration in serum and urine in diabetes

NO_2/NO_3 in serum was lower in patients with uncomplicated diabetes compared with control group ($p=0.002$). In diabetic nephropathy, serum NO_2/NO_3 did not differ from the control group. In diabetic nephropathy, NO_2/NO_3 in urine was lower when compared to patients with uncomplicated diabetes and controls



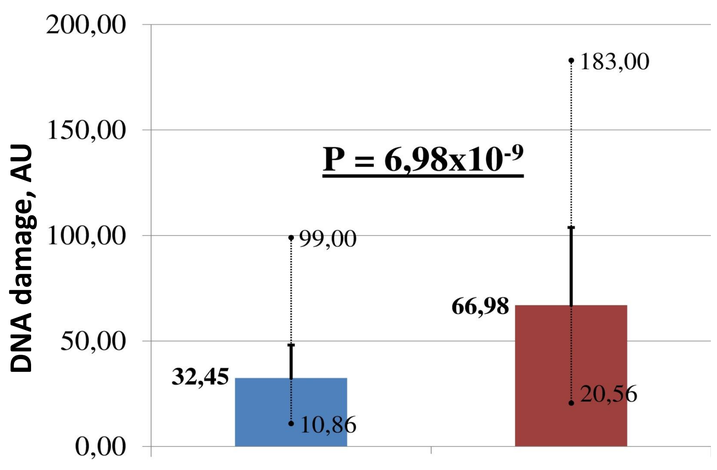
NO_2/NO_3 in serum of healthy subjects, patients with uncomplicated type 1 diabetes and diabetic nephropathy (DN) (mkM). + - p< 0.05 vs control.



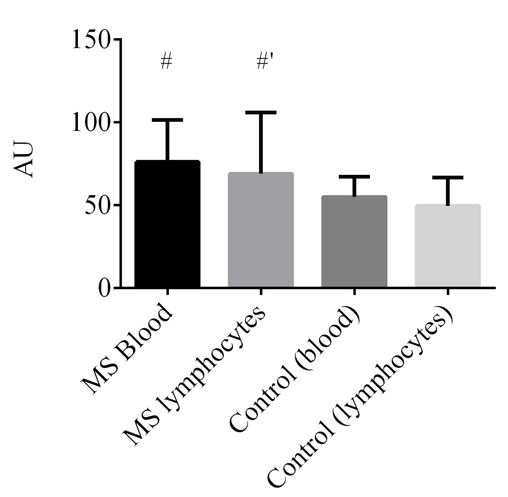
NO_2/NO_3 in urine of healthy subjects, patients with uncomplicated type 1 diabetes and diabetic nephropathy (DN) (mkM). + - p< 0.05 vs control.

Single-strand DNA breaks

Level of single-strand DNA breaks was higher in patients with T1D and MS, compared to healthy subjects. The level of DNA damage in whole blood and lymphocytes of the same group was similar



Single-strand DNA breaks (arbitrary units) in wholeblood of healthy subjects and patients with type 1 diabetes



Single-strand DNA breaks (arbitrary units) in wholeblood and lymphocytes of healthy subjects and patients with MS

Conclusion

- Multiple sclerosis and type 1 diabetes are associated with increased NO in wholeblood
- Type 1 diabetes is associated with decreased NO_2/NO_3 in serum
- In diabetic nephropathy, NO and NO_2/NO_3 accumulate in blood and NO_2/NO_3 excretion in urine is reduced
- Multiple sclerosis and type 1 diabetes are associated with increased level of single-strand DNA breaks

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