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The antioxidative capacity of ENADA-NADH in humans

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Introduction

NADH is a strongly reducing agent and has been suggested to act – possibly by indirect means – as important antioxidant. Only recently direct antioxidative effects of NADH were demonstrated, and NADH was suggested as major antioxidant in mitochondria [1].

We investigated antioxidant effects of NADH both *in vitro* and *in vivo*. We employed various techniques because data on antioxidative effects of diverse compounds depend strongly on the methods used.

Methods

Thirty-seven healthy volunteers with 18 to 30 years of age were chosen for the study and divided into two groups. The study was performed as a placebo-controlled double-blind study.

One group was administered 20 mg NADH daily for four weeks; the second group received a placebo preparation. Blood was drawn at the beginning and at the end of administration. After six week wash out phase the groups were changed, and NADH as well as placebo were administered again for four weeks.

Reaction of NADH with nitrogen-centered radicals was measured by two different methods. A commercial test kit based on the formation of 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] ($ABTS^+$) radicals was employed (Randox). We performed this assay in kinetic mode measuring the lag time of radical formation instead of a single time point measurement. By the second technique, quenching of the stable radical diphenylpicrylhydrazyl (DPPH) was determined. From the concentration dependence of radical scavenging the concentration of 50% scavenging (IC_{50}) was calculated.

Reaction with superoxide was measured with a device allowing photochemical generation of superoxide and luminometric measurement of its formation (Photochem system, Analytik Jena). By this method the lag time of radical formation is determined.

Oxidation of low density lipoprotein (LDL) was performed by incubation LDL (0.3 mg/ml) in the presence of 2mM AAPH, 10 μM EDTA and in the presence or absence of 50 μM NADH or ascorbic acid, respectively at 37°C. Oxidation was monitored by measurement of diene formation (OD_{234}).

The prooxidative potential was measured by investigating the influence of NADH on hydroxylation of salicylic acid in the presence or absence of copper ions in comparison to ascorbic acid. Salicylic acid was incubated with copper ions in the presence or absence of the compound. Hydroxylation products of salicylic acid were quantified by HPLC with electrochemical detection.

Malondialdehyde in plasma was measured after reaction with thiobarbituric acid using HPLC with fluorescence detection (excitation wavelength 527 nm, emission wavelength 550 nm). For calibration 1,1,3,3-tetrametoxypropane was used.

Plasma glutathione in whole blood was measured using a commercial test kit (Oxis Research). Autoantibodies against oxidized LDL were determined with serum samples by a time-resolved fluorescence immunoassay. The immunoassay developed by Cerne et al. was used with certain modifications [2].

Carbonyl content of plasma proteins was measured according to Gerber et al. [3].

Results and discussion

NADH reacts with ABTS* radicals and shows distinct lag times in the kinetic assay. Is capacity to scavenge ADTS* is identical to that of ascorbic acid and trolox Figure 1 shows the behavior of NADH versus ascorbic acid.

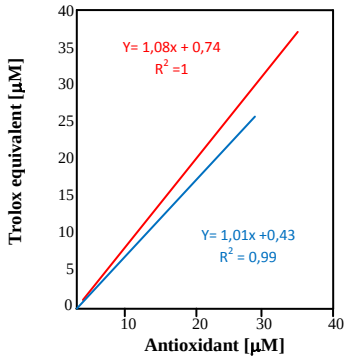

Figure 1: The reaction of NADH (blue) and acerbate (red) with the $ABTS^+$ - radical

NADH also reacts with DPPH. The concentration for half maximal scavenging (IC_{50}) of 50 μM DPPH is 20 μM. For GSH and ascorbic acid we found IC_{50} values of 120 and 8 μM, respectively (Figure 2).

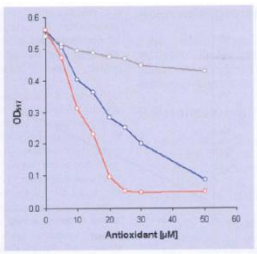

Figure 2: The reaction of NADH (blue), ascorbate (red) and glutathione (grey) with DPPH

On the other hand, NADH is not a scavenger of superoxide. While increasing concentrations of ascorbic acid gave increasing lag times of radical formation, NADH even at a tenfold concentration shows no lag time (Figure 3):

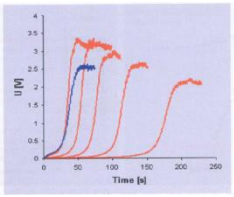

Figure 3: The reaction of NADH (blue; 20nmol) and ascorbate (red; from left to right 0, 0.25, 0.5, 1 and 2 nmol) with the superoxide radical anion

When LDL is oxidized in vitro induced by proxyl radicals NADH reveals an antioxidant effect identical to ascorbic acid during the first 90 minutes. However, after 90 min ascorbic acid has no further effect while NADH is still acting antioxidatively (Figure 4):

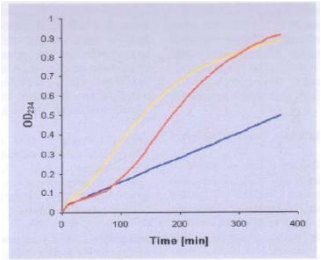

Figure 4: Diene formation during AAPH*-induced LDL oxidation in the presence of ascorbate (red) and NADH (blue) versus control (yellow)

When NADH is incubated in the presence of salicylic acid in PBS, no hydroxylation of salicylic acid is observed and only 12 % of NADH is lost after 9 h (Figure 5).

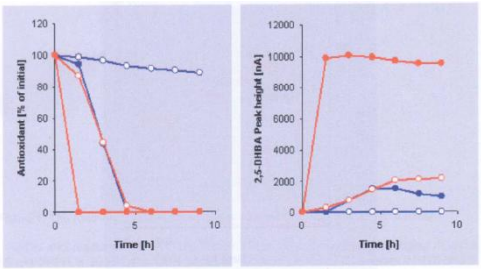

Figure 5: Incubation of salicylate with 500μM NADH (blue) or ascorbate (red) in the absence (open symbols) or presence (full symbols) of Cu2+ (10μM). Left: Consumption of antioxidant; Right: Formation of 2,5-dihydroxybenzoic acid (DHBA).

After addition of copper ions NADH is consumed rapidly and some hydroxylation occus. On the other hand, when ascorbic acid is used instead of NADH, hydroxylation products are found even without addition of copper ions and on the presence of Cu^{2+} hydroxylation is enhanced dramatically. In conclusion, NADH appears to be a particularly useful antioxidant which may be less prone to become prooxidative under certain conditions.

In vivo, we investigated a variety of different variables in a group of 37 medical students who voluntarily participated in a double-blind placebo controlled study of oral intake of ENADA-NADH. Particularly in smokers, NADH exerted some reduction of the concentration of malondialdehyde (P = 0.080) and particularly of oxidative stress-induced protein carbonyl modification (P = 0.018): by means of a newly developed and very sensitive assay method we could demonstrate that initially elevated protein carbonyl modification levels steadily decreased and, within the study period, approached levels of non-smokers. No effect of NADH in the antioxidative capacity measured by the glutathione test, Photochem test, Randox test and autoantibodies against oxidized LDL was found. In fact, most subjects investigated showed quite normal values of all these parameters throughout the timecourse of the study (young, healthy volunteers). Nevertheless, this pilot

investigation provided some evidence that smoking produces elevation of malondialdehyde and protein carbonyl modification levels and these pathological signs could be improved by NADH. Therefore, we presently investigate the possibility that in longstanding heavy smokers more strongly pronounced pathological values of these oxidative stress-related markers might be seen and, therefore, beneficial effects of NADH may be much easier to detect.

We conclude that NADH appears to be a potent antioxidant, both in vitro and *in vivo*, with particularly favourable properties: while its antioxidative capacity in most systems approaches that of ascorbic acid, it prooxidative effects may be much less expressed in comparison with Vitamin C.

Acknowledgement

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References

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