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Workshop report

Nicotinamide – biological actions and therapeutic potential in diabetes prevention

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Nicotinamide, a derivative of the B_3 vitamin nicotinic acid, has been shown to prevent both chemically-induced [1] and spontaneous development [2] of diabetes mellitus in animal models of Type 1 (insulin-dependent) diabetes. In addition, nicotinamide preserves residual beta-cell function and enhances beta-cell regeneration in partially pancreatectomized rats [3]. Furthermore, nicotinamide promotes growth of cultured human fetal islet cells [4].

These observations have led to clinical pilot trials which try to prevent Type 1 diabetes in first degree relatives of patients with Type 1 diabetes. These trials suggest that nicotinamide can prevent, or at least delay, the onset of Type 1 diabetes in man. Data also suggest that nicotinamide is less effective if treatment begins close to the onset of diabetes, i.e. in individuals with high islet cell antibody (ICA) titre and low first phase insulin response (FPIR). Hence, there is evidence to suggest that nicotinamide could protect human beta cells, particularly if the drug was given early in the pre-diabetic stage of the disease process.

To clarify the effect of nicotinamide in the prevention of Type 1 diabetes in man, a prospective, randomized, placebo-controlled study is needed. A multi-centre study involving 18 European countries, Israel, and Canada is planned. This **E**uropean **N**icotinamide **D**iabetes **I**ntervention **T**rial (ENDIT) will investigate the impact of daily oral administration of nicotinamide in first degree relatives at increased risk (ICA > 20 Juvenile Diabetes Foundation (JDF) units) of progressing to clinical disease. For this workshop of the International Diabetes Immunotherapy Group (IDIG) a number of diabetologists, pharmacotoxicologists, and basic scientists from the cancer/DNA-repair field were invited to discuss different aspects of nicotinamide action.

Generation of free radicals resulting in impairment of respiratory enzymes and DNA strand breaks have been implicated in the immune-mediated beta-cell destruction [5]. Nicotinamide is involved in a number of injury and repair mechanisms centered on DNA. The covalent binding of the nuclear enzyme poly(ADP-ribose) polymerase (PADPRP) to nucleoproteins is an important part of the repair mechanism of damaged DNA. PADPRP is thought also to participate in other cellular processes, including proliferation, differentiation, transformation, and gene rearrangements and transpositions. The process of DNA repair is powerfully stimulated by the presence of DNA damage and strand breaks. PADPRP uses nuclear NAD as a substrate to catalyse poly(ADP-ribosyl)ation of nuclear acceptor proteins. In this process the dinucleotide moiety of NAD is used to make poly (ADP-ribose).

Nicotinamide plays a dual role in this process. It is an essential part of NAD, thus increasing the NAD-pool, but it also inhibits the action of PADPRP. The inhibitory effect of nicotinamide on PADPRP in vitro, i.e. in cell-free systems, has a concentration giving 50 % inhibition (IC_{50}) of 31 μmol/l and in vivo an IC_{50} of 100 μmol/l [6]. The peak serum-level obtained when 1.2 $g \cdot m^{-2} \cdot day^{-1}$ of a slow-release preparation of nicotinamide is administered orally, as proposed in the ENDIT protocol, will not be higher than 100–120 μmol/l (Petley et al., Southampton, UK). It is at present not known whether serum levels of nicotinamide will reflect intracellular levels. Furthermore, it is not known whether the inhibitory effect on PADPRP or the increasing effect on the NAD-pool will dominate in this dose-range.

Therefore, the crucial question seems to be whether partial PADPRP inhibition is detrimental over the proposed 5-year intervention period planned in the ENDIT protocol. This may depend upon the concentrations of nicotinamide present and the degree of DNA damage. If one considers cells with either low or high degree of DNA damage, and low or high concentrations of nicotinamide, the following combinations are possible. Small amounts of DNA damage will set the ribosylation reaction in motion, depleting NAD which is replenished from the small amount of nicotinamide present. In the case of small amounts of DNA damage and large concentrations of nicotinamide, the ribosylation is inhibited, cellular NAD-pool is high, but DNA repair may be prolonged resulting in increased liability to mutation.

Large amounts of DNA damage and small concentrations of nicotinamide lead to activation of ribosylation, massive NAD depletion and cell death. With large amounts of DNA damage a high concentration of nicotinamide may inhibit DNA repair and prevent cell death, maintaining cytoplasmic NAD, but with long-term risk of large numbers of mutations. It depends on the concentration of NAD and the severity of cell injury whether nicotinamide will assist repair both of DNA and acute cell damage, or whether large amounts of NAD will inhibit DNA repair with a potential for long-term carcinogenic effects. In the presence of free oxygen-derived radicals the O_2^--scavenger effect of nicotinamide reduces the accumulation of $OH^-\cdot$ which could result in DNA strand breaks. Thus, nicotinamide may also prevent DNA damage. The general impression amongst participants in the workshop was that 1.2 $g \cdot m^{-2} \cdot day^{-1}$ of nicotinamide will probably supply NAD without critical inhibition of DNA repair.

Against this background the risk assessment for high dose nicotinamide was carefully reviewed (McLean and Gale, London, UK). The proposal of the ENDIT protocol is to use 1.2 $g \cdot m^{-2} \cdot day^{-1}$ of nicotinamide (corresponding to 2–3 g/day). This is approximately 100–150 times recommended daily intake (20 mg/day). The pharmacokinetics of nicotinamide are not well characterized but it seems to be well absorbed with a reported half-life as long as 9 h. Tissue distribution is generally unknown but uptake into liver and erythrocytes has been reported. Nicotinamide is mainly excreted in the urine.

In short- and long-term studies nicotinamide has been found to have low toxicity. No carcinogenic effects have been demonstrated by long-term feeding of nicotinamide to rats (1 % in drinking water). There is no evidence of teratogenic effects. There have been some clinical reports of adverse effects in humans taking large doses of nicotinamide for a long time, in particular of readily-reversed increment in serum levels of liver enzymes.

In rats nicotinamide also affects growth. One percent of nicotinamide in the diet given to rats resulted in impaired growth. This effect was reversed with the addition of methionine. Since humans, like rats, carry out the methylation reaction of nicotinamide resulting in the excretion of methylated derivatives, they may as well be liable to methionine deficiency if fed large amounts of nicotinamide during growth. However, rats offered 1 % of nicotinamide in the food have a daily intake of approximately 1 $g \cdot kg^{-1} \cdot day^{-1}$, corresponding to 40 times the dose proposed in the ENDIT protocol.

Rodents offered food or drinking water with the foul-tasting nicotinamide added have reduced food intake. It cannot be excluded that the reduced food intake per se is responsible for the reduction in growth. There are no reports on the impact of nicotinamide on growth in humans.

Nicotinamide is also implicated directly in preserving beta-cell function during the immunological process leading to Type 1 diabetes. Studies presented have demonstrated macrophages (Kolb, Düsseldorf, FRG, and Hauschildt, Freiburg, FRG) and islet cells (Andersen, Gentofte, Denmark) as producers of the toxic free radical nitric oxide (NO), and that nicotinamide inhibited this production. Furthermore, a number of in vitro studies indicate that nicotinamide protects beta-cell function and morphology against the deleterious effects of free NO radicals/interleukin-1β(IL-1) (Andersen, Gentofte, Denmark, and Buscema, Catania, Italy). This suggests that nicotinamide may interfere in the autoimmune process in Type 1 diabetes by decreasing the production of NO in macrophages in the insulitis process and/or directly in islet cells.

The concentration of nicotinamide proven to be effective in these in vitro studies is approximately 1,000 times higher than peak plasma levels in humans. The clinical relevance of these findings is not known. However, it cannot be excluded that low concentrations of nicotinamide for long periods of time (years) might produce the same effects seen in vitro using high concentrations and short-time exposure (days).

Nicotinamide from various suppliers contains up to 4 % of different impurities including nicotinic acid (Jørgensen, Bagsværd, Denmark). Crystallization can reduce the impurities to undetectable levels, and highly purified nicotinamide is as potent as commercially available nicotinamide in protecting isolated islets of Langerhans against IL-1 induced inhibition of insulin secretion. It was concluded that impurities should be removed when nicotinamide is used for prolonged periods in pharmacological doses. Pharmacokinetic studies in man of regular and sustained-release oral preparations of nicotinamide showed that the peak levels are 2.5-fold higher and the bioavailability is 1.5-fold higher following regular nicotinamide compared to sustained-release preparations (Petley et al., Southampton, UK). Both preparations were detectable in the blood up to 6 h after oral intake. The high peak concentration following administration of regular nicotinamide might inhibit PADPRP, however it is not known whether or not this is advantageous. The pharmacokinetics of the sustained-release, highly purified nicotinamide to be used in the ENDIT are under investigation.

Nicotinic acid decreases insulin sensitivity in man, an action inexpedient in pre-diabetic patients. However, recent studies suggest that intake of nicotinamide for one week in "ENDIT dosages" does not influence insulin secretion and insulin sensitivity in healthy subjects (Mahon et al., London, Canada).

Elliott (Auckland, New Zealand) reported upon an open, population-based, clinical trial in ICA-positive schoolchildren using nicotinamide for prevention of Type 1 diabetes. In a cohort of 80,000 children (5–7 years old), 32,000 were offered an ICA test. Only 20,000 were tested; 150 of these had ICA over 20 JDF units and were prescribed supplementation with nicotinamide in a dose of 1 g/day. At the follow-up after 2.3 years, no cases of Type 1 diabetes mellitus were found among the 20,000 ICA tested, but five cases among the 12,000 "intended to test" but not tested. In the 48,000 "not intended to test" there were 47 cases of Type 1 diabetes after a follow-up of 4.3 years. Eisenbarth (Boston, Mass., USA) showed data indicating that nicotinamide is useless in pre-diabetic pa-

tients close to diabetes onset (ICA over 80 JDF units and low FPIR). These data raised the question whether there is a "point of no return" close to diabetes onset where nicotinamide is without effect. The answer may eventually be extracted from the result of the ENDIT study.

The impact of nicotinamide has also been investigated in newly-diagnosed diabetic patients. However, the results are conflicting. In some studies nicotinamide administration had a beneficial effect on the incidence of remission after one year (Harter et al., Nice, France), but other studies have not been able to confirm this observation (Ilkova et al., Istanbul, Turkey, and Yilmaz et al., Istanbul, Turkey). More consistently, it was reported that nicotinamide increased the insulin secretion capacity after one year. The clinical relevance of this phenomenon is doubtful if nicotinamide has no impact on the period of remission. These data also support the hypothesis that there is a "point of no return".

Altogether, the data presented at the workshop left the impression that the nicotinamide intervention of Type 1 diabetes as proposed in the ENDIT protocol is relevant. Utilising current knowledge long-term treatment of prediabetic individuals with high doses of nicotinamide should not be harmful.

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