Phytopharmaceuticals in Cancer Chemoprevention and Therapy

Chapter 12: NADH in Cancer Prevention and Therapy

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1. Biological functions of NADH

NADH is the abbreviation for **N**icotinamide **A**denine **D**inucleotide **H**ydride. NADH is also known under a number of other synonyms such as:

Diphosphopyridine nucleotide, reduced form
Adenine-D-ribose-phosphate-phosphate
D-ribose-nicotinamide, reduced form
Cozymase, reduced form
Coenzyme 1, reduced form
Codehydrogenase, reduced form
Nadide, disodium salt, reduced form

NADH is present in every living cell where it catalyzes more than a 1000 bio-chemical reactions. The most important biological functions of NADH are the following:

1.1 NADH is the cellular fuel for energy production
1.2 NADH plays a key role in DNA and cell damage repair
1.3 NADH enhances the cellular immune system
1.4 NADH is the most potent antioxidant

1.1 NADH is the fuel for cellular energy production

All living cells require energy to stay alive. Without energy, a cell dies because the energy production represents the essential prerequisites for every living cell.[1]

How is energy produced in the cell? NADH reacts with oxygen to produce in a cascade of biochemical reactions water and energy. This energy is stored in form of the chemical compound adenosine triphosphate, abbreviated ATP. NADH itself is produced from amino acids, sugars and lipids via the citric acid cycle. One molecule of NADH yields three molecules of ATP and the more NADH a cell has available, the more energy it can produce.[2] The amount of NADH a cell contains depends on the amount of energy it requires. Heart muscle cells, which have to contract themselves every second for entire life-time which is 86400 times a day, contain 90 mcg of NADH per gram tissue. Brain and muscle cells contain 50 mcg.[3] One third of all the energy produced by our body is used up by our brain.

1.1.1. NADH increases the mitochondrial membrane potential

The British Nobel laureate Peter Mitchell postulated that energy in the mitochondria is formed by a gradient of electric charge between the outer and the inner side of the mitochondrial membrane. The higher the level this electric potential, the more energy is produced. Researchers in China could demonstrate that incubating cells with NADH leads to an increase in the mitochondrial membrane potential[5] implying more energy output.

1.1.2. Extracellular NADH increases intracellular ATP production in heart cells

A most recent study has shown that NADH can increase the biosynthesis of ATP inside the cell. Isolated single heart cells were incubated with NADH. An increase of ATP inside the cell was found by two independent methods.[4] This observation provides convincing evidence that NADH can penetrate the cell membrane and increase the cellular energy level in form of ATP.
If the cell has more energy, it can live longer and can perform its functions better. The consequences and implications of these findings are remarkable. Heart cells get more energy by NADH, hence their strength and capacity is higher. People with heart problems can benefit from NADH. After a heart attack some areas in the heart may be damaged and hence not functioning but still alive.

By supplying these cells NADH, they may get more energy to repair the damage and will become functional again.

The same principle may work in the brain. After a stroke, certain areas in the brain are not nurtured by blood as the circulation is blocked. These brain parts may be still vital but not functioning. By offering them NADH they get more energy and may regain their functionality. A number of anecdotal cases of stroke patients treated with NADH did show improvement of their symptoms even weeks after the event.

If NADH leads to an increase in energy in isolated heart cells it should also work in other tissues such as the kidney, the liver, the pancreas or the lung.

It was some kind of dogma that NADH does not pass the cell membrane because it is too hydrophilic and too labile to penetrate into the intact cell. The study out-lined has convincingly shown by two independent methods that NADH can in-crease ATP formation and energy

production in isolated heart cells.[4] In doing so, NADH must penetrate the cell membrane to get to the point of action, the mitochondria. NADH is also taken up by cells lacking mitochondria such as erythrocytes. If you incubate human red blood cells with NADH a decline of the extracellular NADH and an increase in ATP (= energy) in these cells is observed (Hallström et al, personal communication). The consumption of NADH by blood cells correlates (indirectly) to the level of ATP.[6] In other words, if blood cells consume a lot of NADH, the ATP level in these cells is low.

Highly conditioned athletes are assumed to have a high energy level in their muscles and blood cells, respectively. Hence their blood cells consume only a low amount of NADH when incubated with it. Blood cells from elderly or sick people consume considerably more NADH than athletes. However, when athletes are tested after a marathon run or a long distance cycling, their blood cells consume NADH in an amount comparable to that of old people. These observations were made with a newly developed blood test.[6]

This new patented test has been named ENMA (**E**xtracellular **N**ADH **M**etabolization **A**ssay). It has an enormously broad application range.[7] It can be used not only for controlling the training performance of athletes but also in the surveillance of patients in terms of energy recovery after a heart-attack, a stroke, cancer treatment or rehabilitation.

1.2 NADH plays a key role in DNA and cell damage repair

The DNA in the nucleus is well protected by histones and other macromolecules. Nevertheless, it can be damaged by exposure to various agents such as radiation, UV light, ozone, free radicals, carcinogens and toxins such as cytostatic drugs some of which are themselves carcinogenic. These potentially harmful agents do react with the chromosomes. If the DNA is affected and damaged by one of these agents the genetic material will be altered. Replication of altered, defective DNA causes changed features in the newly divided cells if provided cell division can still occur. The greater the DNA damage the more extensive alterations in cells and tissue occur.[8] Genetic damage is the biochemical basis for a number of chronic diseases such as cancer[9,10], rheumatoid arthritis, immunodeficiences and arterio-sclerosis.[9,10] Hence it is imperative that our genetic material remains unaltered in order to guarantee that any new progenitor cell developing after cell division is identical to their parent cells. If the DNA is altered by physical or chemical agents, the newly developing progenitor cells may be different from their mother cells and will not function in the originally programmed way.
In order to avoid the fatal consequences of DNA damage, mammalian cells have developed a system which is able to repair alterations of their genetic material. This so-called DNA repair system needs NADH to gain full functionality.[12,13] Therefore, the more NADH you have in your body, the better the DNA repair system functions and the better you are protected from potentially developing diseases.

The exposure of cells to DNA-damaging reagents can trigger a wide range of cellular responses involved in the regulation of gene expression and cell-cycle progression, stimulation of DNA repair and programmed cell death.[14,15] These processes are important for maintaining normal growth, anti-mutation, damage repair and functional activity of cells. However, due to the unspecificity of chemotherapeutic drugs for the cancerous target cells, many normal cells get damaged as well causing severe, sometimes fatal adverse reactions. The question is how can normal cells be protected from the cytotoxic effects of chemotherapeutic agents? How can we stimulate the repair system and promote normal cellular responses after chemotherapy? The mechanism involved in repairing DNA-damaged cells exposed to cytostatic has been investigated in many clinical studies.[14-18] Whether the reduced form of the coenzyme

nicotinamide adenine dinucleotide (NADH) can be used to protect cells from DNA-damage has never been considered until recently. Previous studies in our laboratory have found that NADH can stimulate biosynthesis of endogenous cell factors, and can rescue cells from apoptotic damage by triggering production of the bcl-2 oncogene proteins[19].

The effect of NADH on DNA repair was investigated on PC12 cells, damaged by doxorubicin. PC12 cells were incubated in medium with and without NADH before and after exposure to the DNA damaging agent doxorubicin. The changes of the cell proliferation genes (c-myc, c-erbB-2) the apoptosis inhibition gene bcl-2 and p53 (tumor suppressor gene), cell apoptosis inhibition gene bcl-2 and p53 (tumor suppressor gene), cell apoptosis gene (c-fos) and the proliferating cell nuclear antigen (PCNA) were investigated using a cytotoxicity assay and immunofluorescence flow cytometric analysis.

Doxorubicin induced DNA damage in PC12 cells by inhibiting the expression of the cell proliferation genes and by triggering apoptotic processes in the cells. This was shown by down regulating the expression of c-erb-2, c-myc, bcl-2 and up regulating the expression of PCNA and c-fos of the PC12 cells.[20]

NADH did not only increase the resistance of PC 12 cells to the doxorubicin induced DNA damage but did also repair the damage partially. NADH promoted survival and differentiation by regulating the c-myc oncogenes protein. Furthermore it supported the process of DNA repair by regulating the expression of p53, bcl-2 on the PC 12 cells damaged by doxorubicin. NADH also down regulated expression of the cell apoptosis gene c-fos on the PC12 cells.

The expression of c-erB-2 oncogene protein and PCNA on the PC12 cells did not show a significant change in the group of cells incubated with NADH in comparison to the group incubated with medium alone. In addition, an abnormal proliferation effect of NADH on PC12 cells has not been observed in these experiments.

As a consequence of these findings, NADH may be considered as a therapeutic adjunct for cancer patients to protect them against the general toxic effects of sub-stances such as doxorubicin or cisplatin by stimulating the DNA repair system and by promoting normal cellular biosynthetic responses after chemotherapy. NADH seems to exhibit a chemo preventive effect.

Drug-induced apoptosis is dependent on the balance between cell cycle check-points and DNA repairing mechanisms. Doxorubicin is a DNA-damaging cytotoxic drug, which is found to accumulate in the nuclei of damaged cells. Increased accumulation of cellular doxorubicin is accompanied by apoptosis.[14,15,21] Experiments indicate that the inhibition rate of PC12 cells correlates with the concentration of doxorubicin in medium and with time of exposure of the cells to the toxic environment.

The cytotoxicity of doxorubicin for PC12 cells occurs not only in the phase of acute exposure but also in the lag phase.

Apoptosis induced by doxorubicin is accompanied by the down-regulation of the expression of the oncogenes proteins c-erb-2 and c-myc, the anti-apoptotic gene proteins (bcl-2), p53 tumor suppressor protein and upregulation of the expression of PCNA[16] and c-fos.

These genetic changes occur not only in the early phase of the apoptosis induced by doxorubicin, but can also happen in the lag phase, when the damaged PC12 cells are incubated with new medium after removing the old doxorubicin containing medium. DNA damage and

activation of c-fos oncogene seem to be the major pathways of inducing apoptotic damage of PC12 cells. NADH can partially rescue cell activity of PC12 cells from DNA damage induced by doxorubicin. Cell damage repair is a complex biological process in which a number of reactions are involved. NADH is an essential component of enzymes necessary for many metabolic reactions in the cell including energy production. It plays a crucial role in triggering biological antioxidation and in regulating the expression of membrane glycoprotein receptors.[22,23] Previous studies have shown that NADH can rescue cells from apoptosis caused by inhibition of the mitochondrial respiratory chain induced by chemotherapeutic agents such as Rotenone, and simultaneously can increase the production of endogenous biological factors necessary for proper functions. In addition, cell cycle progression of PC12 cells is observed.[15,19]

When the apoptotic rate of PC12 cell was 82.2%, the rate of cells repaired by NADH was only 3.1%. After recovery incubation for 48 hours, the expression of c-erbB-2 oncogene proteins and PCNA on the PC12 cells did not show a significant increase in the group treated with NADH in comparison to the control.

The change of cerbB-2 oncogene happening in the acute damage phase of the PC12 cells is difficult to be rescued by incubation with NADH or medium. However, the upregulation of c-fos oncogenes protein in the acute damage phase can be significantly down-regulated by incubation with NADH for 48 hours. This suggests that NADH rescues PC12 from doxorubicin induced damage not only by repairing the DNA but also by increasing energy production in these cells.

Programmed cell death is an energy dependent biochemical regulated process that is the result of the expression of a number of genes. The roles of several gene and gene families such as Bcl-2/bax, P53, c-myc, c-jun, c-fos, considered to be critical for apoptosis have recently been described in different cell lines.[24,25] Many reports suggest that a rather complex genetic and molecular mechanism is involved in the process of apoptosis. It could also be triggered either by increased or by reduced gene expressions as well as by biochemical reactions not necessarily connected to altered gene expression.[14,24-26]

Observations from our studies provide evidence that complex molecular events are involved in the apoptotic process of PC-12 cells induced by doxorubicin. After recovery incubation of PC-12 cells with NADH for 48 hours, the positive ratio and amount of c-erbB-2 expressed on PC12 cells did not show an increase in comparison to the control with medium alone. The positive ratio of c-myc was not altered, but the amount of c-myc expressed on the vital PC12 cells was significantly upregulated 47.7% and 52.9% in comparison to the acute damage phase and the group with medium alone. This suggests that regulating the expression of c-myc on PC12 cells may be involved in the DNA repair of PC12 cells damaged by doxorubicin. Although the exact function of c-myc remains largely unknown, its activation has been implicated in the induction of cell proliferation and differentiation has been implicated in the induction of cell proliferation, and differentiation. Some reports have also indicated that the c-myc oncogenes protein acts as sequence-specific factor that serve to regulated gene expression in normal cellular growth and differentiation and as a common intracellular transducer which pro-mote G_0 to G_1 transition. They may also be involved in the regulation of programmed cell death (27,28,29,30).

In the processes of cell DNA damage repair, bcl-2 and p53 are the two of the most important proteins encoded by the bcl-2 gene and p53 tumor suppressor gene. Wild-type p53 can suppress cell proliferation and slow DNA synthesis and block transition from G1 to S phase of the cell cycle (30,31,32). Bcl-2 is a proto-oncogene and the most important inhibitor of apoptosis. Expression of bcl-2 may interfere with the apoptotic process mediated by the APO-

1/Fas antigen and TNF receptor. Probably the ratio of bcl-2 and p53 determines how the cell responds to DNA-damaging agents. Current research indicates that expression of bcl-2 in Pheochromocytoma cells is associated with that of the c-myc oncogene protein.[33-35] Over expression of the proto-oncogene bcl-2 might block p53-induced apoptosis and inhibit p53 functional activity.[36] In our experiment, in which we investigated the effect of NADH on the recovery of PC12 cell from DNA-damage, the ratio of expression of p53 and bcl-2 on PC12 cells was down-regulated by 91.9% and 98.8% after exposure of the cells with doxorubicin. After recovery incubation of the cells in medium containing NADH for 48 hours, the ratio of vital PC12 cells was upregulated by 3.1% and p53 tumor suppressor protein expressed on the vital cells down-regulated by 36.7%. However, the amount of bcl-2 expressed on the vital PC12 cells was found to be upregulated by 12.7% in comparison to the control group (medium alone). These findings suggest that NADH can not only pro-mote survival and differentiation of cells by regulating the c-myc oncogenes protein, but also support the process of DNA repair by regulating the expression of p53 tumor suppressor protein and proto-oncogene protein bcl-2 on the PC12 cells damaged by doxorubicin.

Cisplatin is one of the most frequently used drugs for chemotherapy of cancer. It damages the cell membrane, the mitochondria and the nucleus not only from cancer cells but from all normal non cancerous cells as well. The consequences are the so-called side effects of chemotherapy such as hair loss, gastrointestinal problems (vomiting, dizziness etc.)

Preincubation of cells which have been damaged by cisplatin with NADH prevents the changes induced by cisplatin.[37]

Based on these findings, cancer patients should protect themselves by taking NADH when receiving Cisplatin, doxorubicin or other cell damaging cytostatic drugs.
NADH is also involved in transcriptional pathways important for development, cell cycle regulation and transformation.

The co repressor CtBP (carboxy-terminal binding protein) binding to cellular and viral transcriptional repressors is regulated by the nicotinamide adenine dinucleotides NAD and NADH, with NADH being two to three orders of magnitude more effective.[38]

The best-characterized target promoter for CtBP in mammalian cells is probably the E-cadherin gene.[39,40] Loss of E-cadherin expression in tumors correlates with metastasis, invasion and poor clinical prognosis.[41,42]

It has been shown that CtBP-mediated repression of the E-cadherin promoter is enhanced by hypoxia.[38] NADH may alleviate the hypoxic state by stimulating oxy-gen uptake into the cell. It has been shown that the oxygen uptake in the muscle of highly conditioned athletes increases after taking the stabilized orally absorb-able form of NADH.[42] In addition, NADH seems to be a sensor of blood flow needed in brain muscle and other tissues.[43] Increasing blood flow removes lactate and augments delivery of nutrients and oxygen for energy metabolism.

1.3.   NADH stimulates cellular immune functions

The cellular immune response in humans is based on the activities of the T-lymphocytes, the B-lymphocytes and the macrophages. Macrophages have the capability for direct elimination of allergenic entities such as bacteria, viruses and other foreign tissues. The first step in the elimination of bacteria is the perturbation of the plasma membrane of macrophages. As a consequence the metabolic activity including oxygen consumption is markedly increased. Most

of oxygen is converted to superoxide and hydrogen peroxide.[44] This phenomenon, known as "metabolic burst" appears to be the first and most critical step leading to the destruction of the invading foreign organism. During this metabolic burst and the cytotoxic activity induced in the macrophages high amounts of NADH are needed and used. Hence the immune defense mechanism of white blood cells is fueled by NADH. Furthermore it has been shown that NADH stimulates the biosynthesis of interleukin-6 (IL-6). Peripheral human blood leucocytes when incubated with NADH significantly stimulate the release of IL-6 a dosage dependent manner.[45]

Beside a number of other functions, IL-6 has been reported to protect neurons from degeneration the mechanism of which has not yet been elucidated. If IL-6 protects neurons it may protect other cells as well.[46,47]

1.4. NADH is the most powerful antioxidant

An antioxidant is a substance which acts against oxidation. The opposite of oxidation is reduction. Compounds with a high reduction potential exhibit a strong anti-oxidative power. NADH, the reduced form of Coenzyme 1 has the highest reducing power as a single biological molecule. Only molecular hydrogen has a higher reduction potential but does not exist in living cells. Biological antioxidants are pre-sent in all living cells to protect the cell and its membrane from destruction by free radicals.[48] Free radicals are molecules with an unpaired electron. Hence they are extremely reactive. They interact with many compounds in human cells, in particular with the lipid-containing structures such as the cell membrane. In doing this, they violate the integrity of the cell wall causing leakage and release of essential cellular components which usually results in cell death.[49] Free radicals have been shown to be involved in the development of cancer[50] coronary heart disease atherosclerosis, diabetes, neurodegenerative disorders and autoimmune diseases.[51,52] Free radicals are formed in human cells by agents knocking out electrons from a molecule. These agents can be X-rays or other forms of high-energy radiation such as the one used for radiotherapy of cancer. Small amounts of free radicals are also produced in normal cells by metabolic reactions. However, mammalian cells possess a defense system to protect them from being irreversibly damaged.[53] This system is called "antioxidative protection shield". The first and most important antioxidant component in this system is NADH, because it has the highest reduction potential of any compound in the cells.[54] A measure for free radical formation and lipid peroxidation concomitantly are the thiobarbituric acid reactive species (TBARS) determination. In a study using spontaneous hypertensive rats (SHR), it has been found that the renal TBARS were significantly lower (1.9 nmol/MAD/100 mg tissue) in the rats fed with 5 mg NADH orally as compared to the control animals (3.5 nmol/MDA/100 mg tissue). MDA is the abbreviation for malondialdehyde which is formed from the breakdown of polyunsaturated fatty acids. NADH also reduced total cholesterol and LDL-cholesterol significantly as well as the blood pressure.[55] One of the conclusions the authors deduced from these findings in that NADH may appear to become a useful agent for preventing and treating cardiovascular risk factors. The antioxidative effect of NADH was also investigated in humans. When LDL cholesterol is oxidized in vitro induced by per-oxyl radicals NADH reveals an antioxidant effect identical to ascorbic acid during the first 90 minutes.[56] However, after 90 min the effect of ascorbic acid ceases whereas NADH continues to act antioxidatively. Hence the antioxidative potency of NADH appears to last much longer than that of ascorbic acid. In a double-blind placebo controlled study 37 human subjects were given ENADA®-NADH (4 tablets of x 5 mg NADH) or placebo tablets for 4 weeks. NADH caused a reduction of malondialdehyde level and also in the (oxidative stress-induced) carbonyl modification of proteins particularly in smokers. A steady decrease of the initially elevated protein carbonyl modification levels of smokers was observed which approached the levels of

non-smokers within the study period of 4 weeks. This observation implies that ENADA®-NADH may have a preventive or even curing effect on tissues damaged by cigarette smoking.

2. ENADA – the stabilized orally absorbable from of NADH

NADH can be regarded as biological form of hydrogen. Hence it is a very reactive compound. NADH is very unstable and becomes easily degraded by air, water, humidity, acids and oxidizing agents such as sugars. Even in solid state NADH reacts with lactose, the most common filler of tablets. In 1987, NADH has been used intravenously for treatment of Parkinsonian patients. The beneficial effects in improving the disability of the PD patients were remarkable.[57] The challenge was to transpose the i.v. form of NADH into an oral (tablet) form. After yearlong research a Gaelic formulation was developed in which NADH was stable for at least 2 years a prerequisite for registration as an ethical drug. For this special formulation of a stabilized orally absorbable form one of the authors (G.B.) received worldwide patents.[58,59] The brand name for the patented stabilized orally absorb-able form of NADH is ENADA®. Numerous controlled clinical studies have been performed with ENADA® since its development 1993.

3. Bioavailability of ENADA® – NADH

The stabilized form of NADH when taken orally is absorbed in the small intestine. Studies have shown that NADH passes the intestinal mucosa undegraded by passive diffusion.[60] In a further study it could be demonstrated that ENADA®-NADH passes the blood-brain barrier.

When rats were fed with two tablets ENADA-NADH 5 mg an increase of the NADH level in the brain cortex was observed after 20 minutes of intake measured by la-ser-induced fluorescence.[61]

Using a pulsed N-2 laser combined with a fiber-optic probe and photomultipliers the NADH fluorescence was measured in the brain cortex of rats. After intraperitoneal application of NADH (50 mg/kg) an increase in the intensity of the cortical NADH fluorescence of about 18 % was observed for approximately 30 minutes compared to the fluorescence intensity in the control group. Neither NAD+ (the oxidized form of NADH) at concentration of 50 mg/kg nor nicotinamide (50mg/kg) did show any effect on the NADH fluorescence in the cortex for the entire measurement of 120 minutes.

Following oral application of NADH (2 tablets of ENADA 5mg NADH = 51 mg/kg) the cortical fluorescence intensity was increased by about 20% compared to the control group.[61] The results of this study provide convincing evidence that NADH given orally increases the amount of NADH in the brain. To achieve this, ENADA®, the stabilized orally absorbable form of NADH has to pass the blood brain barrier.

4. ENADA® – NADH - a protector against chemotoxicity and radiation

Cytostatic drugs such as Cisplatin and Doxorubicin are used in chemotherapy of cancer. These drugs trigger a wide range of cellular responses involved in the regulation of gene expression and cell cycle progression and programmed cell death.

As these cytostatic drugs are not specific neither for cancerous nor for normal cells, the latter may get damaged causing severe sometimes fatal adverse reactions. Studies from the authors have shown that NADH can stimulate the biosynthesis of endogenous cell factors and can rescue cells from apoptotic damage by triggering production of the bcl-2 oncogene proteins.[62] In

the process of cell dam-age repair bcl-2 and p53 are the two of the most important proteins encoded by the bcl-2 gene and p53 tumor suppressor gene. Wild type p53 can suppress cell proliferation and can slow DNA synthesis and thus block transition from G1 to S phase of the cell cycle.[63] Bcl-2 is a proto-oncogene and the most important inhibitor of apoptosis. Current research indicates that expression of bcl-2 is associated with that of the c-myc oncogen protein.[64] In studies performed by the authors it was found that Doxorubicin down regulated the expression of p53 and bcl-2 in PC 12 cells by 91.9% and 98.8% respectively. Incubation of the damaged cells by NADH promoted survival and differentiation by regulating the c-myc oncogene protein. NADH also supported the process of DNA repair by regulating the expression of the p53 tumor suppressor protein and the proto-oncogene protein bcl-2.[62] Similar results were obtained when Cisplatin was used as cell-damaging agent.[37]

In the same publication the effect of NADH on cells damaged by radiation was reported. When PC12 cells were exposed to radiation in a dose given routinely as radiotherapy of cancer, 90% of vital cells were destructed.

Incubation of the destructed cells with NADH induced a repair process. Of the 90% damaged cells more than half of it could be repaired and gained full functionality (Zhang J.R., personal communication).

A number of cytostatic drugs including Cisplatin are carcinogenic and can cause cancer. NADH is able to protect cells from the carcinogenic effects of these chemotherapeutic agents. Hence ENADA®, the stabilized oral form of NADH, may present a safe, non-toxic, biological supplement for prevention of cancer.

5. The safety of ENADA-NADH

The stabilized orally absorbable form of NADH (ENADA®) is a nutritional supplement available in the U.S. since 1995 and in the E.U. since 1997. Based on the patented formulation of this supplement, a number of clinical trials have been launched to prove scientifically that ENADA is effective. In order to get these studies started, an Investigational New Drug Application (IND) which was filed with the Food and Drug Administration (FDA). For FDA approval it has to be documented that ENADA (the stabilized oral form of NADH) is safe. For this reason, the maxi-mum tolerated intravenous dose (MTD) of ßNADH (reduced form of beta-nicotinamide adenine dinucleotide) in beagle dogs was elucidated. The maximum tolerated dose (MTD) of ßNADH in dogs was found to be 500 mg NADH per kg per day. In other words a 10 kg heavy dog will tolerate 5 grams of NADH.[65] In addition the oral form of NADH (ENADA) was tested in beagle dogs. 150 mg/kg/day were given form 14 days. This corresponded to 30 regular (5 mg) ENADA tablets filled in 2 gelatin capsules (15 ENADA tablets per capsule). This high dose was selected because it was considered to be the maximum amount which could be practically administered repeatedly over 14 days. All dogs survived the treatment and no adverse reactions or side effects were observed.

The dogs treated with ENADA showed no changes in comparison to the control animals regarding laboratory safety parameter and organ and tissue pathology.
150 mg /kg bodyweight means 1500 mg for a 10 kg beagle dog. 1500 mg of ENADA correspond to 300 ENADA 5mg tablets per day. This is a dose which beagle dogs tolerate without any side-effect.[65]

In addition to the MTD findings a study for potential chronic toxicity of ENADA was performed in rats. Rats were given 1 tablets ENADA (5mg NADH) per day for 26 weeks. No changes in

laboratory parameter and in tissue and organ pathology were observed.[66] 5 mg for a rat weighing about 330 grams corresponds to 15 mg per kg bodyweight or 1050 mg of NADH for a 70 kg heavy human subject. 1050 mg NADH correspond to 210 tablets of ENADA (5mg NADH) which are tolerated without side effects when given for 26 weeks (6.5 months). Based on these safety data ENADA-NADH can be generally regarded as safe and the FDA gave the permission for two clinical trials in the U.S. in between 2 weeks after application.

6. <u>ENADA-NADH as therapeutic concept for certain human cancers</u>

NADH has been shown to inhibit the growth of murine fibro sarcoma and human laryngeal carcinoma cells in vitro.[67] Based on these findings and the various bio-logical functions the stabilized oral form of ENADA® has been used as treatment for certain types of cancer.
A number of anecdotal cases will be described in the following in which ENADA-NADH was used as anti-cancer therapeutic approach.

Case 1: 48 year old male suffering from a small cell bronchial carcinoma. The diagnosis was made by MRT (magnetic resonance tomography) and verified by histopathological examination of biopsy specimen. The size of the tumor was 6 to 8 cm in diameter in Sept. 2001 when the patient visited one of the authors (G.B.) .The report of the University of Amsterdam indicated the tumor was inoperable due to its localization very close to the mediastinum. The patient had received radio-therapy followed by chemotherapy before he came for a visit to one of the authors (G.B.). The patient was recommended to take 4 tablets ENADA 5mg NADH per day. He was already taking Selenium, vitamin C and vitamin E. In January 2002 the size of the tumor was, as verified by MRT, to be the size of a cherry. The therapy with NADH was continued. In July 2002 a MRT report of the University of Amsterdam no tumor has detectable.

Case 2: Female, aged 63, August 1989, operation for invasive duct carcinoma. One year later multiple liver and bone metastases detected. Four therapy cycles, according to the CMF diagram, further increase of liver and bone metastases. Pain only reducible with the strongest analgesics. Since January 1991 NADH, initially three times a week 12.5 mg, intravenously, and then after four weeks Parenteral therapy change to NADH orally, 5 mg every day. April 1991 radiological detection of metastasis regression, some foci greatly reduced in size and some completely disappeared. The oral NADH therapy was continued. A check in 1991 using CT scanning revealed a further marked regression of the liver metastases and the bone metastases were virtually undetectable. Patient free from pain and no longer requires analgesics. The serum concentration of CA15.3 dropped from 65.0 (Jan. 1991) to 24 (Aug. 1994).

Case 3: Male, aged 59, colon carcinoma three years earlier, 1990 sonographic and radiological detection of multiple liver metastases of cherry to plum size. Two chemotherapy cycles, Myleran or Endoxan unsuccessful, liver foci increased in size. December 1990 start of therapy with NADH, initially 12.5 mg intravenously three times a week and after four weeks change of therapy to NADH orally, 5 mg, every day. March 1991 sonographic detection of reduced liver foci size. June 1991 Check by CT scanning and sonography revealed an almost complete disappearance of the metastases in the liver. Subjectively the patient feels extremely well. The tumor marker CEA was 110 (Dec. 1990) and declined to 22 by November 1994.

Case 4: Female, aged 52, three years earlier quadrantectomy due to invasive scirrhous carcinoma of the breast. In January 1990 vertebral metastases were detected, April 1990 liver metastases were discovered by ultrasonic examinations. Therapy with Novaldex lead to no regression of the metastases. Also no response to a therapy cycle according to the CMF-diagram was observed. November 1990 intravenous administration of NADH 12.5 mg every

other week was stated. After four weeks it was changed to NADH orally, 5 mg every day. Two months after the start of NADH therapy clear regression of liver metastases, as well as disappearance / reduction of vertebral metastases. Liver metastases were greatly reduced or foci no longer detectable. CEA and CA 15.3 were 45 and 92 by April 1990. The last control in October 1994 showed CEA to be 14 and CA15.3 to 18.5.

Case 5: Male, aged 66, February 1990 parvicellular bronchial carcinoma was diagnosed, multiple foci in both pulmonary lobes were formed. Cytostatic therapy with methotrexate and Endoxan led to no regression. In October 1990, NADH was administered parenterally (10 mg intravenously) every other day. Radiographic check in 1991 revealed the remission of the neoplastic foci both as regards to number and size. NADH therapy was continued with 10 mg orally every day. A check in May 1991 by CT scanning confirmed a further reduction of tumor foci in both pulmonary lobes.

Case 6: Male, aged 72, November 1990 diagnosis of a tumor mass in the liver (8-10 cm in diameter). In summer 1993 multiple lung metastases of various sizes have been found in the CT scan. Patient denied surgical intervention as well as chemo- or radiotherapy. Since spring 1994 he is taking one tablet of NADH every day. Control examination by X-ray and computer tomography showed no increase of the lung metastases and a reduction of the liver mass with indications of formation of necroses in the centre of the tumor. The patient feels subjectively well and has no pain. The lung cancer associated tumor marker CYFRA 21-1 was 35 be-fore NADH therapy (April 94) and 21 in December 1994. The carcinoembryonic antigen CEA levels were measured to be 67 in April 94 and 28 in December 94.

Case 7: Female, aged 55. February 1992 lymph node metastases of a poorly differentiated mammary carcinoma were detected in left neck region. The CA 15.3 value was 37.0, the CEA level 13.5 and the TPS 145 in March 1992. The primary tumor could not be localized. The patient denied chemo- and/or radiotherapy. She was given 5 mg NADH every day. A year later the previous palpable lymph node metastases had disappeared. The tumor marker tests CA 15.3, CEA and TPS were 15.0, 8.0 and 95 respectively in July 94. Computer tomography and bone scan did not show any metastases (June 94).

For the time being we can only speculate on the mechanism of action of NADH in stabilizing or reducing certain cancers.

One possibility could be the function of NADH as DNA repairing agent. Cancer cells have a DNA which is altered in comparison to the original cells from which the carcinoma cells develop. If NADH is given to cancer patients the content of NADH in the cancerous cell increases. The more NADH a cell has available the better the DNA repair system works and the alteration of the genes may be reverted to normal. Another possibility may be derived from the energy increasing function of NADH. As mentioned earlier in this paper, the intracellular level of ATP can be increased by incubating the cells with NADH. With more energy cancerous cells increase their capacity of the biosynthesis of macromolecules in particular proteins, glycol-proteins and glycolipids. These substances play a major role on the cell surface in regulating proliferation and differentiation. With more NADH and ATP in the cancerous cells proliferation may be halted and differentiation processes may be induced. These assumptions remain to be elucidated in further studies.

Acknowledgment

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