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33 NADH in Cancer Prevention and Therapy

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33.1 BIOLOGICAL FUNCTIONS OF NADH

NADH is the abbreviation for nicotinamide adenine dinucleotide hydride. 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

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Coenzyme 1, reduced form
Codehydrogenase, reduced form
Nadide, disodium salt, reduced form

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

NADH is the cellular fuel for energy production
NADH plays a key role in DNA and cell damage repair
NADH stimulates cellular immune functions
NADH is the most potent antioxidant

33.1.1 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 producing water and energy. This energy is stored in the form of the chemical compound adenosine triphosphate (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 (86,400 times per day) for an entire lifetime, contain 90 μg of NADH per gram of tissue. Brain and muscle cells contain 50 μg.[3] One-third of all the energy produced by our body is used by our brain.

33.1.1.1 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 of this electric potential, the more energy is produced. Researchers in China demonstrated that incubating cells with NADH leads to an increase in the mitochondrial membrane potential,[4] implying greater energy output.

33.1.1.2 Extracellular NADH Increases Intracellular ATP Production in Heart Cells

A recent study has shown that NADH can increase the biosynthesis of ATP inside the cell. Isolated single heart cells were incubated with NADH, and an increase of ATP inside the cell was found by two independent methods.[5] This observation provides convincing evidence that NADH can penetrate the cell membrane and increase the cellular energy level in the 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 be alive. If these cells are supplied with NADH, they may get more energy to repair the damage and 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 outlined has convincingly shown by two independent methods that NADH can increase ATP formation and energy production in isolated heart cells.[5] 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. 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 after long-distance cycling, their blood cells consume NADH in an amount comparable with that of old people. These observations were made with a newly developed and patented blood test known as ENMA (extracellular NADH metabolization assay).[6] 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.

33.1.2 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, ultraviolet (UV) light, ozone, free radicals, carcinogens, and toxins such as cytostatic drugs, some of which are themselves carcinogenic. These potentially harmful agents can 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 provided that cell division can still occur. The greater the DNA damage, the more extensive are the alterations that can occur in cells and tissue.[8] Genetic damage is the biochemical basis for a number of chronic diseases such as cancer,[9,10] rheumatoid arthritis, immunodeficiencies, and arteriosclerosis.[9,10] Hence it is imperative that our genetic material remain unaltered in order to guarantee that any new progenitor cell developing after cell division is identical to its 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 for repairing 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 cytostatics 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-erb-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 by up-regulating the expression of PCNA and c-fos of the PC12 cells.[20]

NADH not only increased the resistance of PC12 cells to the doxorubicin-induced DNA damage, but also partially repaired the damage. NADH promoted survival and differentiation by regulating the c-myc oncogene proteins. Furthermore, it supported the process of DNA repair by regulating the expression of p53 and bcl-2 on the PC12 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 proteins and PCNA on the PC12 cells did not show a significant change in the group of cells incubated with NADH in comparison with the group incubated with medium alone. In addition, no abnormal proliferation effect of NADH on PC12 cells was observed in these experiments.

As a consequence of these findings, NADH can be considered as a therapeutic adjunct for cancer patients to protect them against the general toxic effects of substances 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 chemopreventive effect.

Drug-induced apoptosis is dependent on the balance between cell-cycle checkpoints and DNA-repair mechanisms. Doxorubicin is a DNA-damaging cytotoxic drug that accumulates 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 oncogene proteins c-erb-2 and c-myc, the anti-apoptotic gene proteins (bcl-2), p53 tumor suppressor protein, and up-regulation 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 h, the expression of c-erb-2 oncogene proteins and PCNA on

the PC12 cells did not show a significant increase in the group treated with NADH in comparison with the control. The change of c-erb-2 oncogene that occurs during the acute damage phase of the PC12 cells is difficult to be reversed by incubation with NADH or medium. However, the up-regulation of c-fos oncogene protein in the acute damage phase can be significantly down-regulated by incubation with NADH for 48 h. This suggests that NADH rescues PC12 from doxorubicin-induced damage not only by repairing the DNA, but also by increasing energy production within these cells.

Programmed cell death is an energy-dependent biochemically 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 PC12 cells induced by doxorubicin. After recovery incubation of PC12 cells with NADH for 48 h, the positive ratio and amount of c-erb-2 expressed on PC12 cells did not show an increase in comparison with 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 up-regulated 47.7% and 52.9%, respectively, in comparison with 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. Some reports have also indicated that the c-myc oncogene protein acts as a sequence-specific factor that serves to regulate gene expression in normal cellular growth and differentiation and as a common intracellular transducer that promotes G0 to G1 transition. It 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 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] The bcl-2 gene 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] Overexpression 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 h, the ratio of vital PC12 cells was up-regulated by 3.1%, and the p53 tumor suppressor protein expressed on the vital cells was down-regulated by 36.7%. However, the amount of bcl-2 expressed on the vital PC12 cells was found to be up-regulated by 12.7% in comparison with the control group (medium alone). These findings suggest that NADH can not only promote survival and differentiation of cells by regulating the c-myc oncogene 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 of cancer cells, but from all normal noncancerous 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 that might be 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 corepressor 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 oxygen uptake into the cell. It has been shown that the oxygen uptake in the muscles of highly conditioned athletes increases after taking the stabilized, orally absorbable 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.

33.1.3 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 allogenic 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 in a dosage dependent manner.[45]

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

33.1.4 NADH Is the Most Powerful Antioxidant

An antioxidant is a substance that acts against oxidation. The opposite of oxidation is reduction. Compounds with a high reduction potential exhibit a strong antioxidative 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 this does not exist in living cells. Biological antioxidants are present 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, usually resulting 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 that 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 –- called the “antioxidative protection shield” — to protect them from being irreversibly damaged.[53] The first and most important antioxidant component in this system is NADH, which has the highest reduction potential of any

compound in the cells.[54] The thiobarbituric acid reactive species (TBARS) determination provides a measure of both free-radical formation and lipid peroxidation. In a study using spontaneous hypertensive rats (SHR), it was been found that the renal TBARS were significantly lower (1.9 nmol MDA/100 mg tissue) in the rats fed with 5 mg NADH orally as compared with the control animals (3.5 nmol MDA/100 mg tissue). MDA (malondialdehyde) 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 is that NADH may be 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 peroxyl radicals, NADH reveals an antioxidant effect identical to ascorbic acid during the first 90 min.[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 (four tablets of 5-mg NADH) or placebo tablets for 4 weeks. NADH reduced malondialdehyde levels as well as 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, ultimately approaching the levels of non-smokers within the study period of 4 weeks. This observation implies that ENADA–NADH may have a preventive or even curative effect on tissues damaged by cigarette smoking.

33.2 ENADA: THE STABILIZED ORALLY ABSORBABLE FORM OF NADH

NADH can be regarded as a biological form of hydrogen. Hence NADH is a very reactive compound, 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 was used intravenously (i.v.) for treatment of patients with Parkinson's disease (PD). The beneficial effects in improving the disability of the PD patients were remarkable.[57] The challenge was to transpose the intravenous form of NADH into an oral (tablet) form. After yearlong research, a galenic 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 absorbable form of NADH is ENADA. Numerous controlled clinical studies have been performed with ENADA since its development 1993.

33.2.1 Bioavailability of ENADA–NADH

When taken orally, the stabilized form of NADH 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 was 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 min of intake as measured by laser-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 min compared with the fluorescence intensity in the control group. Neither NAD+ (the oxidized form of NADH) nor nicotinamide (both at concentrations of 50 mg/kg) showed any effect on the NADH fluorescence in the cortex for the entire measurement period of 120 min.

Following oral application of NADH (two tablets of ENADA [5 mg NADH] = 51 mg/kg) the cortical fluorescence intensity was increased by about 20% compared with 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 and orally absorbable form of NADH, had to have passed the blood–brain barrier.

33.2.2 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 to either cancerous or normal cells, the latter may be damaged during chemotherapy, 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 damage repair, bcl-2 and p53 are 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] The bcl-2 gene 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 oncogene protein.[64] In studies performed by the authors, it was found that doxorubicin down-regulated the expression of p53 and bcl-2 in PC12 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 damaged. Incubation of the damaged cells with NADH induced a repair process. More than half of the damaged cells could be repaired and gained full functionality (Zhang, personal communication).

A number of cytostatic drugs, including cisplatin, are carcinogenic. 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, nontoxic, biological supplement for prevention of cancer.

33.2.3 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 (IND) application was filed with the Food and Drug Administration (FDA). For FDA approval, it must be documented that ENADA (the stabilized oral form of NADH) is safe. For this reason, the maximum tolerated intravenous dose (MTD) of βNADH (reduced form of beta-nicotinamide adenine dinucleotide) in beagle dogs was elucidated. The maximum tolerated dose (MTD) of βNADH in dogs was found to be 500 mg NADH per kg of body weight per day. In other words, a dog weighing 10 kg will tolerate 5 g of NADH.[65] The oral form of NADH (ENADA) was also tested in beagle dogs, who were fed 150 mg/kg/day for 14 days. The drug was delivered in the form of 30 regular (5 mg) ENADA tablets filled in two gelatin capsules (15 ENADA tablets per capsule). This high dose was selected because it was considered to be the maximum amount that 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 with the control animals regarding laboratory safety parameters and organ and tissue pathology. A dose of 150 mg/kg body weight means 1500 mg for a 10 kg beagle dog, and 1500 mg of ENADA corresponds to 300 5-mg ENADA tablets per day. This is a dose that 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, which were given one tablet of ENADA (5 mg NADH) per day for 26 weeks. No changes in laboratory parameters or in tissue and organ pathology were observed.[66] A dose of 5 mg for a rat weighing about 330 grams corresponds to 15 mg per kg body weight, or 1050 mg of NADH for a 70-kg human subject. A 1050-mg dose of NADH corresponds to 210 tablets of ENADA (5 mg 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 permission for two clinical trials in the U.S. about 2 weeks after application.

33.2.4 ENADA–NADH as Therapeutic Concept for Certain Human Cancers

NADH has been shown to inhibit the growth of murine fibrosarcoma and human laryngeal carcinoma cells *in vitro*.[67] Based on these findings and the various biological functions, the stabilized oral form of ENADA has been used as treatment for certain types of cancer.

33.3 CASE STUDIES

The following subsections describe a number of anecdotal cases in which ENADA–NADH was used as an anticancer therapy.

33.3.1 Case 1

The first case involved a 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 September 2001 when the patient visited one of the authors (G.B.).The report of the University of Amsterdam indicated that the tumor was inoperable due to its localization very close to the mediastinum. The patient had received radiotherapy followed by chemotherapy before he came for a visit to one of the authors (G.B.). The patient was recommended to take four 5-mg tablets of ENADA 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, an MRT report from the University of Amsterdam stated that no tumor was detectable.

33.3.2 Case 2

A female, aged 63, underwent an operation for invasive duct carcinoma in August 1989. One year later, multiple liver and bone metastases were detected. Following four therapy cycles, according to the CMF diagram, further increase of liver and bone metastases were observed. Pain was reducible only with the strongest analgesics. Beginning in January 1991, the patient was treated with NADH (12.5 mg) administered intravenously three times a week. After four weeks of parenteral therapy, the patient was switched to an oral regimen, taking 5 mg of NADH every day. By April 1991 radiological detection showed metastasis regression. Some foci were greatly reduced in size, and some completely disappeared. The oral NADH therapy was continued. A check later in 1991 using CT scanning revealed a further marked regression of the liver metastases and the bone metastases were virtually undetectable. The patient was free from pain and no longer required analgesics. The serum concentration of CA15.3 dropped from 65.0 (January 1991) to 24 (August 1994).

33.3.3 Case 3

A male, aged 59, had a colon carcinoma in 1987. In 1990, sonographic and radiological tests detected multiple liver metastases of cherry to plum size. Two chemotherapy cycles, Myleran or

Endoxan, were unsuccessful, and the liver foci increased in size. Therapy with NADH was started in December 1990, initially 12.5 mg intravenously three times a week. After four weeks, the therapy was changed to NADH orally, 5 mg, every day. In March 1991, sonographic detection showed a reduction in the size of the liver foci. In June 1991, a check by CT scanning and sonography revealed an almost complete disappearance of the metastases in the liver. The patient subjectively reported to feel extremely well. The tumor marker CEA was initially 110 in December 1990 and declined to 22 by November 1994.

33.3.4 Case 4

This case involved a female, aged 52. Three years after undergoing a quadrantectomy due to invasive scirrhous carcinoma of the breast, vertebral metastases were detected in January 1990. In April 1990, liver metastases were discovered by ultrasonics examinations. Therapy with Novaldex led to no regression of the metastases. There was also no response to a therapy cycle, according to the CMF diagram. Intravenous administration of NADH (12.5 mg every other week) was started in November 1990. After four weeks, the therapy was changed to NADH orally, 5 mg every day. Two months after the start of NADH therapy, there was a clear regression of liver metastases as well as disappearance/reduction of vertebral metastases. Liver metastases were greatly reduced or foci were no longer detectable. Tumor markers CEA and CA15.3 were 45 and 92, respectively, in April 1990. The last control in October 1994 showed CEA to be 14 and CA15.3 to 18.5.

33.3.5 Case 5

This case involved a male, aged 66. Parvicellular bronchial carcinoma was diagnosed in February 1990, and 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. A radiographic check in 1991 revealed the remission of the neoplastic foci as regards to both 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.

33.3.6 Case 6

This case involved a male, aged 72. In November 1990, a tumor mass in the liver (8 to 10 cm in diameter) was diagnosed. In summer 1993, multiple lung metastases of various sizes were found in a CT scan. The patient denied surgical intervention as well as chemo- or radiotherapy. Beginning in the spring of 1994, he took 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 center of the tumor. The patient subjectively reported to feel well and had no pain. The lung-cancer-associated tumor marker CYFRA 21-1 was 35 before 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.

33.3.7 Case 7

This case involved a female, aged 55. In February 1992, lymph-node metastases of a poorly differentiated mammary carcinoma were detected in the left neck region. The CA15.3 value was 37.0, the CEA level was 13.5, and the TPS was 145 in March 1992. The primary tumor could not be localized. The patient denied chemo- and radiotherapy. She was given 5 mg NADH every day. A year later, the previous palpable lymph-node metastases had disappeared. The tumor marker tests CA15.3, CEA, and TPS were 15.0, 8.0, and 95, respectively, in July 1994. Computer tomography and bone scan did not show any metastases (June 94).

33.4 MECHANISM OF ACTION OF NADH

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 a DNA-repairing agent. Cancer cells have a DNA that is altered from that of the original cells from which the carcinoma cells developed. 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 chapter, 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, glycoproteins, 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.

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