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Anti-nicotine Vaccination: Where Are We?

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Abstract Nicotine is the main substance responsible for dependence on tobacco-containing products, which have a heavy impact on the public health of developed as well as non-developed countries by being a main etiologic factor for the induction of cardiovascular diseases and tobacco-related cancer. A vaccine against nicotine induces antibodies against the molecule, intercepting the nicotine on its way to its specific receptors. The binding of the antibody to nicotine in turn significantly diminishes the nicotine concentration in the brain shortly after smoking. This approach therefore interrupts the vicious circle between smoking and nicotine-related gratification. The preclinical data of our animal experiments are briefly summarized. At the end of 2003, three companies were in early clinical development of an anti-nicotine vaccine: Xenova (TA-NIC), Nabi (NicVAX) and Cytos (Nicotine-Qbeta). The carrier molecules are recombinant cholera toxin B (TA-NIC), an especially selected carrier protein (Nabi) and a virus-like particle VLP (Cytos). Another carrier is additionally used by Chilka in an advanced preclinical model, which showed superiority to cholera toxin B carrier. Cytos has successfully completed a phase I study with 40 healthy non-smoking volunteers. So far, results of a phase I trial by Cytos have shown no unexpected toxicities and phase II trials have now started in Switzerland (Cytos).

1 Introduction

The carcinogenic effect of tobacco was the most important discovery in the history of cancer epidemiology. In the decade 1990–2000 the estimated global tobacco-related deaths toll reached 3 million per year. In the period 2020–2030, global tobacco-related deaths could exceed 10 million annually. The WHO projects that one in ten people now alive will die of tobacco-related

disease if we cannot change this situation for the better. Morbidity and death due to chronic lung disease, cardiovascular disease and cancer mainly of the lung are well-documented events directly related to the total amount of tobacco use over one's lifetime. Women and adolescents are especially prone to develop tobacco-associated cancer early in life, with half as many pack-years compared to an average male smoker.

Modern tobacco control started in the United States with the mandatory printing of warnings against the health risks of smoking on all cigarette packages in 1965. Radio and television advertising for cigarettes has been banned in the USA since 1971, smoking has been forbidden on public transportation since 1990, and the tobacco industry as a whole has been legally challenged by federal and state governments since 1994.

Why are cigarettes so addictive? Over the years, the following, strongly simplified scheme of the mechanism of nicotine addiction has been developed: Nicotine, a compound naturally occurring in tobacco, is sterically very similar to the ubiquitous signaling molecule acetylcholine. It stimulates a heterogeneous group of nicotinic receptors of the adrenal glands, the neuromuscular gaps, and the brain. Like other dependence-inducing drugs, it increases the dopamine level in the nucleus accumbens of the brain (Fig. 1). It furthermore inhibits the enzymatic catabolism of dopamine [1, 2]. The nucleus accumbens itself is one of the main entrances to dependency. In order to make sure that fundamental activities for survival—such as eating, drinking, or sex—are performed, during evolution the brain has connected those activities with the sensation of satisfaction and pleasure. The so-called "highway of pleasure," conceived for this purpose, connects the nucleus accumbens with the hippocampus, where contextual information is stored, and the cerebral cortex, where pleasure enters consciousness [3]. The subjectively perceived difference between the pleasure of a cold beer after a hot day or an orgasm is apparently the consequence of a different activation of the same circuitry. Recent research demonstrates that mediators other than dopamine also play key roles in the network: glutamate

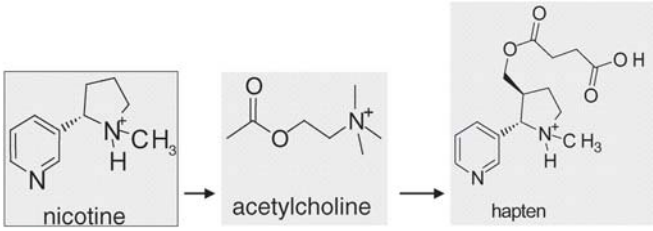

Fig. 1. Structural formula. Illustration of the close steric similarity as well as the similarity in electric change distribution between (−)nicotine and acetylcholine, which is responsible for their binding to the same groups of receptors. The functionalized nicotine hapten was used for coupling (*trans*-3-succinylmethylnicotine) and is shown to the *right*

receptors, for example, are essential in the development of cocaine dependency [4, 5].

2 The Anti-nicotine Vaccination Concept

Why could a vaccine be useful to combat nicotine dependency? In 1972, researchers at the University of Chicago immunized a rhesus monkey against morphine. The animal was shown to be partially protected against heroin (chemically almost identical to morphine), but the authors concluded in their last sentence that subsequent drug challenges could overcome the protective effect: "This blockade has been shown to be dose dependent and it can be overcome by high doses of drugs" [6]. The authors of the same group had published an earlier paper which demonstrates that the high doses of the same conjugate as used in their anti-heroin immunization experiment induces B-cell tolerance, a condition in which no new antibodies against the tolerance-inducing epitopes are produced by the B cells.

The concept of a prophylactic or therapeutic vaccine against drugs of abuse (including nicotine) interrupting the vicious circle between drug consumption and drug-induced stimulation was described for the first time in 1990 by E.H. Cerny [7]. Compared with other medications for smoking cessation, the vaccine concept has some unique advantages: The vaccine effect lasts for years, whereas receptor-antagonist-based medications with their typically short half-life may no longer be taken by the patient once withdrawal symptoms develop. Antibodies do not cross the blood–brain barrier and no secondary effects through interaction with brain receptors are expected. Moreover, having a different mechanism than any other therapeutic group used for smoking cessation, they could be an ideal complement to already established therapies and, as for other vaccines, the expected low or likely nonexistent toxicity as well as a low price may, under certain conditions, allow for a broad preventive application. Like other drugs of abuse, nicotine itself is too small a molecule to be immunogenic in humans and therefore has to be linked to a carrier protein. Useful coupling chemistries for the conjugation of nicotine to a functional group of the carrier protein had previously been developed in the course of the development of radioimmune assays (RIA), which are based on specific antibodies against nicotine [8–10].

3 Material and Methods

Full details can be found in the original publication of the year 2002 [11]. Therefore we only summarize some aspects of this methodological section.

Nicotine in cigarettes is present only as the (−)enantiomer. However, during the high temperature of cigarette combustion up to 11% of the nicotine is transformed into the (+)enantiomer, which has been shown to be pharmacologically active [12, 13]. Therefore, immunization with the racemic mixture is justified in order to maximize the vaccine effect. The strategy for the synthesis of the conjugate followed the derivatization procedure of nicotine as pioneered by Langone and van Vunakis [8, 9].

Immunization Protocols. Female Balb C (Harlan) mice, 7 weeks of age were used for all experiments. Immunizations were performed by subcutaneous (s.c.) injection at the base of the tail of 10, 30, or 100 μg of antigen (nicotine coupled to the carrier protein) in PBS (phosphate-buffered saline) together with 1 mg of Alum as (Alu-Gel S, Serva, Switzerland), in a total volume of 60–100 μl. Depending on the protocol, the animals were boosted at 2- to 4-week intervals with the same amount of antigen in adjuvant by the same route.

For intranasal (i.n.) immunizations, animals under light anesthesia were instilled in both nostrils with 5 μl of conjugate/nostril without adjuvant with the help of a micropipette.

Osmotic Pump. Miniature Alzet osmotic pumps (Alza corporation, USA) model 2004 were implanted into mice subcutaneously on the backs of the animals. The pump has a reservoir of 200 μl, a pumping rate of 0.25 μl per hour, and nicotine was delivered over 4 weeks. The administered dosage was 1.5 mg/kg/day for a mouse of 20 g, which is estimated to correspond to the nicotine-per-weight equivalent absorbed by a person weighting 70 kg, smoking 5 packages a day and absorbing 1 mg nicotine per cigarette.

Challenge with Radioactive Nicotine. The rationale for the calculation of the nicotine equivalent of 2 cigarettes in mice was as follows: a smoker of 75 kg smoking a cigarette absorbs about 1 mg of nicotine. A mouse weights about 20 g and the corresponding quantity per weight is therefore about 300 ng for a cigarette or 600 ng for 2 cigarettes. For practical purposes, 597 ng of non-labeled nicotine and 3 ng of 3H nicotine were injected into the tail vein.

Radioactivity measured in the brain was corrected for the amount of blood present in the brain, considering that 100 g of brain tissue contains approximately 3 ml of blood [14].

Enzyme-Linked Immunosorbent assay. A standard sandwich ELISA (enzyme-linked immunosorbent assay) was used to measure anti nicotine antibodies.

Significant IgA titers were found after vaccination, when given i.n. The IgA antibodies can be detected in the saliva as well as in the serum. The IgA antibodies could be detected in the saliva as well as in the serum.

4 Results of Preclinical Development

Results are best summarized in Figs. 2 and Fig. 3. Significant IgA titers have been found when the vaccine was given i.n.; the IgA antibodies can be detected in saliva as well as in the serum. Figure 2 shows the results of IgA and IgG measurements in both saliva and serum as determined by ELISA using a nicotine bovine serum albumin (BSA) conjugate coated to the solid phase. Each data point presents the result of pooled serum of 5 animals.

Most interesting are the results after nicotine challenge of immunized mice. Figure 3 demonstrates the distribution in the serum and the brain of a 3H-labeled nicotine bolus injected into the tail vein, which corresponds to the nicotine equivalent of 2 cigarettes in mice. The animals are sacrificed 5 min after injection. The mice of group IM1 were immunized 3×i.n. and the mice of group IM2 3×s.c.; serum of 5 animals was pooled for each data point.

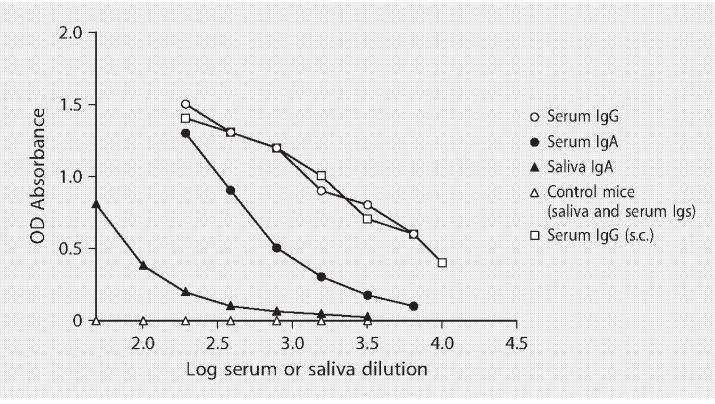

Fig. 2. Intranasal and subcutaneous immunizations. IgG and IgA ELISA results measuring anti-nicotine-specific antibodies in saliva and serum at day 30 after intranasal and subcutaneous immunization with nicotine cholera toxin B (CTB) Berna conjugate. Total doses of 3×30 μg of the nicotine CTB Berna conjugate were applied per mouse in PBS to both nostrils, whereas a control group received 3×30 μg of CTB Berna only. The plates for the ELISA assay were coated with nicotine-BSA conjugate. Two booster instillations were provided on days 7 and 15 post-immunization, and saliva was harvested on day 22 and blood on day 29

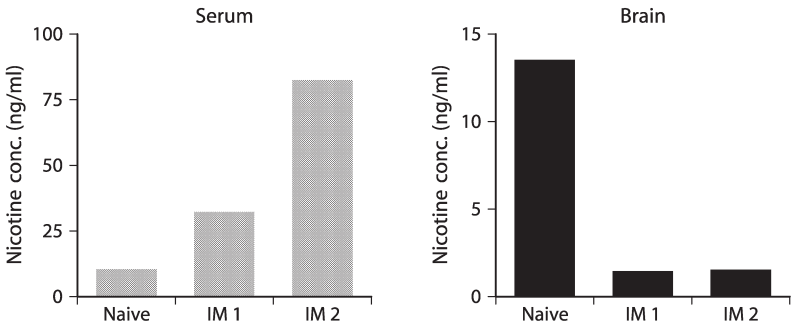

Fig. 3. Nicotine challenge. Distribution in the serum and the brain of tritium-labeled nicotine bolus injected into the tail vein corresponding to the equivalent of 2 cigarettes (600 ng in a mouse of 20 g) in mice sacrificed 5 min after injection. As one would expect, a significant amount of the nicotine is bound in the serum of the vaccinated animals as compared to the naïve animal, but less than 10% of the dose can be found in the brain. (IM1:3×i.n., IM2 3×s.c., serum of 5 animals is pooled for each data point

5 Discussion

Here we describe the preclinical development of an innovative anti-nicotine vaccine for s.c., intramuscular (i.m.), as well as i.n. application, which is in preparation for a phase I evaluation.

The described vaccination approach against nicotine leads to a significant and sustained level of neutralizing antibodies in the animal model and has no apparent toxicity. It leads to an important decrease of nicotine in the brain and therefore breaks the peak inflow of nicotine right after smoking, which is the prerequisite to establish or maintain a nicotine dependency. Intranasal immunization alone produces significant levels of IgA antibodies in saliva and serum as shown in Fig. 2. The efficiency of the intranasal immunization can be deduced from Fig. 3, where the protective effect after i.n. vaccination (IM1) is at least as good as after s.c. (IM2) vaccination. Typically, it takes about 5–6 weeks after the first immunization before high antibody levels are reached in the serum. One may ask if the continuous presence of nicotine in the body as expected in a heavy smoker may interfere with the development of the immune response. A comparison of the mice with and without an implanted nicotine pump, which dispensed the nicotine equivalent of 5 packages of cigarettes a day, answers this question: There is no significant difference in antibody titers obtained between the two groups (Fig. 3).

Figure 3 addresses the question of the reduced nicotine challenge of the brain after vaccination. As one would expect, after 5 min the majority of the radiolabeled nicotine equivalent of 2 cigarettes is bound in the serum of the vaccinated animals as compared to the naïve animals, but only less than

10% of the dose measured in non-vaccinated animals can be found in the brain of the vaccine-protected mice. The same experiment is performed with a challenge of 2.3 ng of radiolabeled nicotine and no signal is detected (less than 1% of control animal, data not shown).

In summary, the concept of neutralization of tobacco-associated nicotine through vaccination against a nicotine conjugate holds promise at the preclinical evaluation stage. Only clinical studies will show if this innovative strategy leads to a powerful tool to overcome and prevent tobacco-associated morbidity and mortality in the future.

6 Where Are We Regarding the Clinical Development?

There are severe ongoing clinical trials in Europe and the USA with the following competing companies:

- Cytos Switzerland with Nicotine-Qbeta (Immunodrug)
- Nabi Inc. USA with Nic-VAX
- Xenova Ltd. (GB) with TA-NIC

In Switzerland, the Cytos product Nicotine-Qbeta (Immunodrug)is now in broad phase II testing after a successful phase I was completed recently. The Cytos Nicotine Immunodrug is shown in Fig. 4 and because of the size of the VLP (virus-like particle) very many antigenic nicotine molecules can

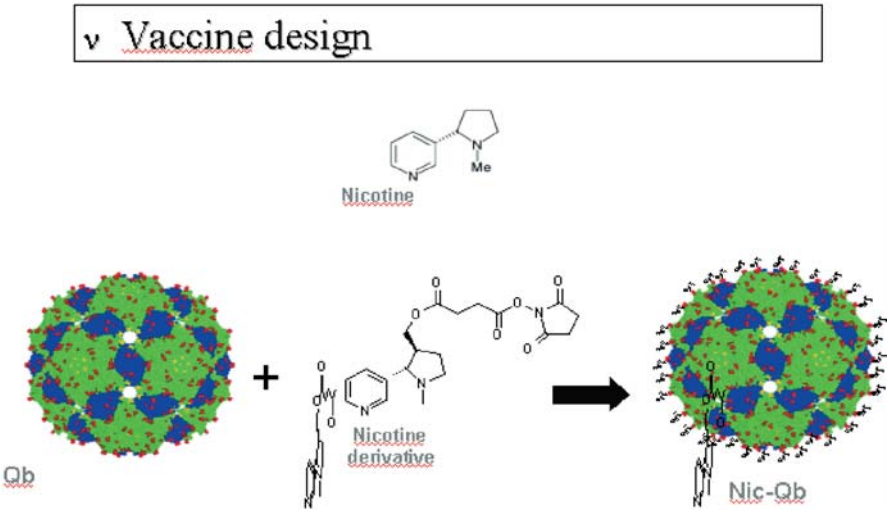

Fig. 4. Structure of the Cytos Nicotine Immunodrug

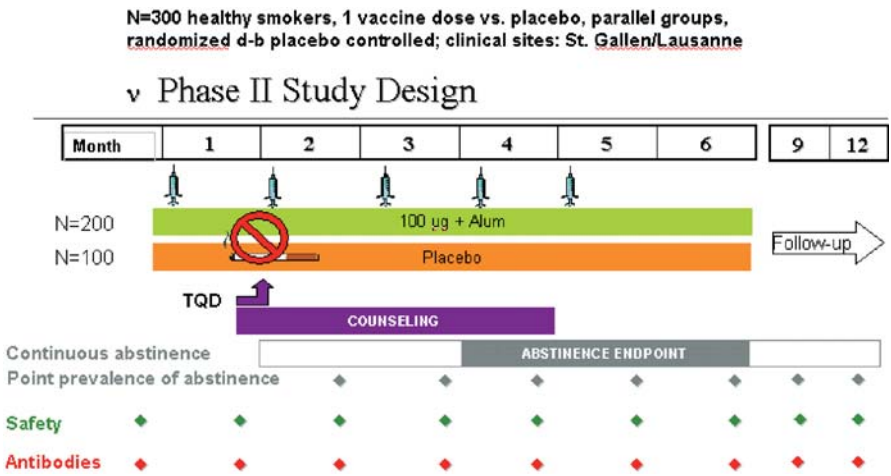

Fig. 5. Outline of the clinical trial with Cytos Nicotine Immunodrug

be bound to its surface. Figure 5 shows the ongoing study outline. Results are expected in early 2005 and the study is ongoing in St. Gallen and Lausanne/Switzerland. Results of the Nic-VAX and TA-NIC vaccination have not yet been published.

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