

APPLICATION OF NICOTINE AND CYTISINE IN NICOTINE ADDICTION TREATMENT

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I. Introduction

The clinical and toxic researches carried out in many countries have proved the enormous harm of nicotine on all systems and organs in human body. Mostly the danger of cardiovascular diseases is underlined (heart-attack, stenocardia, peripheral vascular diseases) respiratory systems diseases (lung cancer, tracheobronchitis, etc) digestion system diseases (gastritis, oral mucosas inflammation, etc.) as well as narcotic type of psychic and physiological dependency.

Nicotine addiction treatment is a slow and complex process in which medicines take important place. Medicamentous therapy should be combined with psychological support for optimising the results achieved. Nicotine addiction treatment gives good results when the so-called nicotine replacement therapy is applied, as the possibility for success is twice as big than with Placebo. [7, 16].

Medicaments used in nicotine replacement therapy exist in different medicinal forms: chewing-gums, trans-dermal therapeutic systems; nasal spray; inhaler. There in a number of commercial preparations containing nicotine (Nicotinell-Ciba Geigy, Nicotrol(R), Nico Derm CQ (R), Habitrol (R), Pstep (R)).

The advantages of the medicamentous therapy based on nicotine are that it suppresses the unpleasant physiological processes arising from abrupt cessation of smoking allowing the patients to focus on behavioral and psychological aspects of smoking [21]. Besides, the effect of nicotine used in medicines comes slower than the nicotine effect from smoking thus the

patients become less dependant by almost immediate effect of the inhaled tobacco smoke. (fig.1)

Fig.1

Concentration of nicotine in plasma

Cigarettes

Spray

Chewing-gum, inhaler/pill

Plaster

Time

Fig.1

Graphic of nicotine level in venous blood after smoking one cigarette and after using different types of medical forms in nicotine replacement therapy after one-day forbearance. [20]

Nicotine replacement therapy must be carefully applies on patients suffering from arrhythmia, angina pectoris and those who have suffered recent myocardial infarct. It is necessary because nicotine might cause unwanted cardio-vascular effects due to increased load of the myocardium as a result of increase cardiac frequency and blood pressure. Nicotine might cause coronary vasoconstriction leading to cardiac Ischemia [5.] The risks of nicotine replacement therapy on patients suffering from cardiac diseases are less compared to the risks from continuous smoking. [6, 14].

Nicotine high toxicity necessitates the search of substances, which have similar effect but are more bearable to the human body. Such an example is the original Bulgarian product Tabex® (Sopharma), developed on the basis of alkaloid Cytisine (N-Holinomimetic) extracted form plant *Cytisus laburnum* L.

Chemistry and distribution of Nicotine and Cytisine

Nicotine and Cytisine belong to the group of alkaloids. They are natural substances of plant origin, which contain one or more nitric atoms in their molecule; they have base character and are physiologically active. This definition cannot be considered as complete and correct, as there are substances in these plants which are isolated and which respond to this characteristic, for example some amines but they are not alkaloids. There are alkaloids with

complex structure but weak pharmacological effect. Nevertheless, the definition given above summarizes the most characteristic properties of this group natural compounds. [1].

1. Nicotine

The history of the chemical identification of Nicotine is described in details [13, 16]. In 1807 Zerioli and in 1808 Vakelin extracted the oil-like liquid from tobacco. In 1828 Poselt and Raiman extracted nicotine from tobacco and in 1843 Melsense described its chemical empiric formula. In 1893 Pickett and Crepeau synthesized nicotine and in 1904 Pickett and Rotshi described nicotine chemical isomers. Not until 1978 Pitner and his associates found the spatial configuration of natural (S-)nicotine.

The experimental application of nicotine has helped significantly our physiological knowledge. Langli and Dickinson [17, 18] noted that high nicotine concentrations block autonomic ganglia thus proving that the autonomic nerves have contact with ganglia. Langli continued using nicotine as pharmacological agent and this significantly expanded the contemporary knowledge on cholinergic synapses [19]. Heimanns and his associates proved that nicotine stimulated breathing through hemo-receptors located in the wall of carotid vein and aorta connected with the carotid sinus and aortic arc. [12].

Nicotine chemical characteristics are well-known. [22]. Pure Nicotine is colorless, water-mixable liquid with characteristic pungent odour. Nicotine boils at 246-247°C. In case of air and light exposure or even if it is stored in a dark place, in a sealed bottle, after some time the colorless or light yellow oily liquid becomes brown in color. The brown Nicotine is as toxic as the pure colorless or light yellow Nicotine. The chemical name of Nicotine is 3-(1-metil-2-pyrolidinil) pyridine; 1-metil-2-(3-pyridyle) pyrolidine; or β -pyridyle- α -N-metilpyrolidine. Its empiric formula is: $C_{10}H_{14}N_{12}$. Its structural formula is:

(R,S) – nicotine

Its molecular weight is 162.23. Its density is d_4^{20} , which is 1.0097. Pure nicotine has a specific spinning angle $[\alpha]_D^{25} = -169^\circ$. When (S)-nicotine is heated to 250°C with tertiary potassium

butoxide it racemates. Therefore nicotine may exist either as (S)- or (R)-form. Below we refer to nicotine (S)- form. Nicotine two isomers are important for the understanding of its molecular pharmacology. They were used in the historical gathering of evidence for the existence of holinergic nicotine receptors.

The basic concept that determines the absorption, excretion, pharmacology and toxicology of nicotine is that its electrically charged and electroneutral forms depend on pH. At 15°C nicotine is two-base due to its pyrrolidine (pKa=7.84) and pyridine (pKa=3.04) nitric atoms. At pH 7.4 and temperature of 37°C about 69 % of pyrrolidine nitric atoms are in ionize form. [8, 9]. Thus the pH in Nicotine solution determines the abrupt change in the level of nicotine protonation. The electroneutral organic bases are lipophilic while the electrically charged organic bases are hydrophilic. Nicotine exists in two forms at pH 7.4. The first passes through lipoprotein membranes but the other – does not. The proportion between nicotine charged and electroneutral forms at pH 7.4 is 2 to 1. This chemical fact determines the important chemical effect of nicotine.

The initial substance for nicotine synthesis is 3-cyanopyridine:

/formula/

3-cyanopyridine

/formula/

/formula/ Nornicotine (R, S)-nicotine

In plants *Nicotiana tabacum* L., *Nicotiana rustica* L., *N. latissima* Mill pyridine and pyrrolidine rings are formed separately and after that they combine:

/formula/

ornitine 1.4-butylenediamine

pyrrolidine

glycerol asparaginic acid nicotinic acid

nornicotine

(R, S)-nicotine

Nicotine belongs to the so-called nicotine group. Other substances that belong to this group are: bi-cycled derivatives including non-condensed pyridine and pyrrolidine rings (nicotine, etc.) or pyridine and piperidine rings (anabasine, etc.) [4].

Folium Nicotianae. Tobacco leaf

Plants. *Nicotiana tabacum* L., *Nicotiana rustica* L., *N. latissima* Mill – Solanaceae family with many varieties, hybrids and genera. *Nicotiana* varieties originate mainly from South America and are grown in a number of countries in Central and South America, South-Eastern Europe. Lesser Asia and etc. [1]

Nicotine and its accompanying alkaloids are found in plants from *Nicotiana* genus. *Nicotiana tabacum* is cultivated throughout the whole world for the production of cigarettes, cigars, pipe and chewing tobacco. Usually nicotine is 2-8 % of the dry leaf quantity though a higher percentage exists in some other plants from *Nicotiana* genus. Some plant varieties belonging to other genera also contain nicotine, for example: *Dubcisia*, *Equisetum*, *Lycopersicum*, *Lycopersicum*, *Lycopodium*, *Sedum* and *Solanum*. Therefore it is not unusual that traces of nicotine are found in some food used by man. [10]. The Nicotine used in industry is a by-product of tobacco industry. By adding calcium or nitric base to filtrated concentrated water extract of tobacco plant parts the alkaloid can be extracted either by means of organic solvent or by means of distillation with water steam. The addition of a drying agent followed by fractionating distillation makes nicotine even purer.

2. Cytisine

Pure Cytisine is white or light yellow crystal powder. Its water solutions have alkaline reaction towards Phenolphthalein. It is easily dissolved in water, spirits and chloroform, it cannot dissolve practically in petrol ether. The chemical name of Cytisine is (1R)-1,2,3,4,5,6-Hexahydro-1,5-methano-8H-pyrido-[1,2-a][1,5]diazocin-8-on. Its empiric formula is $C_{11}H_{14}N_2O$. Its structural formula is:

/formula/
cytisine

Its molecular mass is 190.25. Melting temperature – 152-153°C. Boiling temperature - 218°C. Pure Cytisine has a specific spinning angle $[\alpha]_D^{17} = -120^\circ$. $pK_1 = 6.11$; $pK_2 = 13.08$ [22].

Having in mind the molecular orbital calculations made by Kier it is obvious that there is quaternary nitric atom in molecular configurations of nicotine and acetylcholine, which is negatively charged; it is located at 4.85 ± 0.1 Å and is considered the reason for the nicotine-like activity. The nitric atom in circle C in Cytisine appears at 4.8-4.9 Å of the oxygen atom of pyridine group and is also negatively charged.

Cytisine belongs to the group of hyno-lysidine alkaloids. The basic hetero-cyclic nucleus that forms these alkaloids is hyno-lysidine, which is a double-ring system with common N atom.

Semen Laburni. Golden rain seed

Plant. *Laburnum anagyroides* Medic. (*L. vulgare* Griseb., *Cytisus laburnum* L.) – Fabaceae family. A tree up to 7 m high, with triple leaves and yellow flowers gathered in pendulous multiflorous cluster-like racemes. The fruit is 5-6 cm long bean-like with 3-7 seeds. It originated from Southern Europe where it is cultivated as decorative plant in parks and gardens. [1]

Pharmacological data

1. Pharmacology of nicotine

As a slight base (with $pK_a = 8$) Nicotine has high lypophilicity and quick and complete resorption through oral, pulmonary and gastro-intestinal mucosa. It penetrates easily blood-cerebral barrier and causes central effects. Mainly it stimulates N-holine-receptors in the following order: central nervous system – vegetative ganglia – nerve-muscle synapses. It stimulates M-holine-receptors to a significantly smaller extent.

Taken in moderate doses nicotine causes tachycardia, increase of systolic and diastolic blood pressure, vasoconstriction of renal and blood vessels, and blood vessels dilatation in skeleton musculature stimulates central nervous system, increases gastric secretion and motility and causes slight tremor, releases the anti-diuretic hormone, intensifies salivary secretion.

If taken sharply and in big and toxic doses nicotine causes convulsions, coma, and suppression of breathing. Blood pressure is increased, arrhythmia appears. Paralysis of cross-lined and respiratory musculature occurs. Nicotine has no specific antagonists. [2]

2. Pharmacology of Cytisine

Cytisine is agonist of N-holine-receptors in vegetative ganglia and is referred to the group of ganglia stimulating pharmaceuticals. It stimulates nicotine-sensitive holine-receptors of post-synaptic membrane in vegetative ganglia, chromaffin cells in the medullar part of suprarenal body and sinocarotide reflexogenic zone as a result the respiratory center is stimulated (mainly via reflex way) adrenaline secretion is stimulated from the medullar part of suprarenal body and the arterial blood pressure is increased. After its resorption in gastro-intestinal tract Cytisine plays the part of Nicotine-substituting substance and thus the period of interaction between the nicotine taken and the respective receptors is diminished. On the other hand it leads to gradual decrease and discontinuance of smokers' psychic and physical nicotine addiction.

In different pharmacological experiments a great number of researchers confirm the similarity between pharmacological characteristics of Cytisine and Nicotine described by Dale and Laidlaw (1912). This fact is confirmed by the conclusions of Zackpwski (1938), Anichkov (1937), Dobrev and Paskov (1953), Daleva (1963) [3] etc. They claim that Cytisine is even more powerful as ganglion stimulator than as ganglion blocking agent. This similarity between peripheral effects of Cytisine and Nicotine is more quantitative than qualitative. In experiments with cats and rats (blood pressure examination) or with guinea pig ileum and rat diaphragm comparable effects were received from both preparations as the Cytisine dosages are from $\frac{1}{4}$ to $\frac{2}{3}$ of nicotine dosages.

Considering the effect over central nervous system Cytisine has weaker effect over respiration of anesthetic rabbits in comparison with its effects over peripheral nervous system.

3. Physiological mechanisms of nicotine addiction occurrence and its overcoming

D. Paun and J. Franze examined and analyzed the physiological prerequisites necessary for creating the bad habit of smoking. If a person with low blood pressure and possible low blood sugar concentration inhales a cigarette, nicotine will increase the blood pressure and blood sugar concentration for 20 min by increasing the adrenaline in the blood. If patients with normal blood pressure and blood sugar values smoke a cigarette, the nicotine that gets into the blood causes hormonal and vegetative re-adjustment of the body and if the smoking is terminated it will cause decrease of blood pressure and blood sugar values. That is why nicotine restraining is clinically marked by exhaustion, tiredness, weak concentration and irritability (abstinent behaviour), which causes strong desire to take nicotine. Deprivation syndrome may be soothed by restoration of the normal blood pressure and blood sugar values by means of analeptics, tranquilizers and above all specific preparations such as Cytisine, Lobeline, etc. These specific substances “substitute” nicotine and at the same time act on the same functional receptor constellations.

Pharmacological peculiarities of nicotine acetyl-holine receptors (nAChRs)

Acetyl-holine is one of the first neurotransmitters that have been found (first it was called Vagusschtuff as it was found out that this substance secreted by vagus nerve stimulation, changes the cardiac muscle contractions).

/formula/

Acetyl-holine is synthesised by enzyme holine-acetyl-transferasis, which uses Acetylcoenzyme A and Holine as substrates for forming Acetyl-holine. Holine and Phosphatidylcholine serve as Acetyl-holine synthesis source. Upon secretion Acetyl-holine is metabolised in Holine and acetate by means of Acetyl-holinesterasis and other non-specific esterases. Secretion of Acetyl-holine may be stimulating or inhibiting - depending on the type of tissue and receptor nature it interacts with.

Holinergic receptors are divided in two types – muscarine and nicotine depending on the pharmacological activity of different agonists and antagonists. Muscarine receptors differ from nicotine ones because of their selectivity of agonists: muscarine and nicotine respectively.

1. Nicotine holinergic receptors

Nicotine receptors provoke pharmacologically and physiologically different reactions from muscarine receptors although Acetyl-holine (and other agonists such as carbamilholine) stimulates both types of reaction. Nicotine reactions occur quickly, last for a short time and have stimulating nature. The pharmacology of Nicotine receptors is examined in details, which helps gathering knowledge on the function of ligand-coupled neuro-transmitting receptors. Nicotine receptors are placed in different tissues inclusive in the autonomic nervous system, neuro-muscle synapses and brain of vertebrates. Agonists such as Acetyl-holine and nicotine cause physiological reactions connected with nicotine holinergic activation. Acetyl-holine causes penetration of Na^+ through ligand-dependable ionic channels. Acetyl-holine and carbamilholine stimulates muscarine receptors that is why they should be considered as mixed holinergic agonists. [11].

Many medicines have the effect of endogenic ligands that regulate ionic current through the channels of cytoplasm membrane. Acetyl-holine belongs to the natural ligands of that kind.

Receptors transmit the signal through cytoplasm membrane by means of increase of trans-membrane permeability towards certain ions, which leads to change in membrane electric potential. For example: Acetyl-holine helps for the opening of ionic channel of nicotine holinergic receptor, which allows Na^+ to move to direction of concentration gradient in intracellular space thus provoking depolarization of the relevant local stimulating postsynaptic potential.

Fig. 2. Structure of nicotine Acetyl-holine receptor - ligand-coupled ionic channel. It shows the receptor molecule crossing the cytoplasm membrane with extra-cellular liquid above and cytoplasm below. The receptor consisting of 5 sub-units (2α , 1β -, 1γ -, 1δ -chain) causes opening of central trans-membrane ionic

channel due to the combination of Acetyl-holine with α -sub-units located in the extracellular area.

Nicotine Acetyl-holine receptor is one of the best examined superficial receptors for hormones and neuro-transmitters. It itself is pentamer consisting of 5 polypeptide sub-units (2 α -chains+1 β -, 1 γ - and 1 δ -chain with mol.m. of 43 000 to 50 000). These polypeptides each of which crosses lipid bi-layer, form cylindrical structure with diameter 8 nm. When Acetyl-holine combines with part of α -sub-unit, conformation change occurs which leads to opening of central hydrophilic channel through which the ions of Na^+ penetrate the cell from the extra-cellular liquid. The time between the combination of the agonist with ligand-coupled channel and cell reaction sometimes is measured in milliseconds. The speed of this signal mechanism is very important for the immediate transmission of information by means of the synapse. This fact significantly distinguishes it from the other molecular mechanisms for transmitting signals where this interval may take seconds, minutes even hours. [15].

Conclusion

Nicotine and Cytisine are successfully applied in chronic nicotine addiction therapy. A great number of scientists confirm the similarity between pharmacological characteristics of Cytisine and Nicotine in different types of experiments. This similarity between peripheral effects of Cytisine and Nicotine is more quantitative than qualitative. There are a lot of commercial preparations containing nicotine (Nicotinell-Ciba Geigy, Nicotrol(R), Nico Derm CQ (R), Habitrol (R), ProStep (R)). The advantage of nicotine medicinal therapy is that it suppresses the unpleasant physiological symptoms arising after abrupt cessation of smoking which allows the patients to focus on behavioral and psychological aspects of smoking. Nicotine high toxicity necessitates the search of substances, which have similar effect but are more bearable to the human body. Such an example is the original Bulgarian product Tabex® (Sopharma), developed on the basis of alkaloid Cytisine (N-Holinomimetic) extracted from plant *Cytisus laburnum* L.

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