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Evaluation of anticholinesterasic substances in cigarettes manufactured in Brazil through a human butyrylcholinesterase activity inhibition method

Pesquisa de substâncias anticolinesterásicas presentes em cigarros manufaturados no Brasil por meio de um método de inibição da butirilcolinesterase humana

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Abstract

The objective of this research was to evaluate, through an indirect method, the presence of anticholinesterasic substances in tobacco from cigarettes manufactured in Brazil. Organophosphate and carbamates compounds, which are used in the crops of the tobacco, are the main substances with this characteristic. From each one of the ten main cigarette labels, the tabacco had been separated and extracted anticholinesterasic substances using the method described by Leite (1980) and the n-hexane as solvent. After the preparation of the extract, of the blood was separated the plasma for the determination of the activity of butyrylcholinesterase (BChE) using the method described by Dietz et al (1973).The enzyme activity was measured in the presence of the extracts of each tobacco extract. An significative average inhibition of 20.16%, ($p < 0.001$).

Keywords: Organophosphate. Carbamates tobacco. Butyrylcholinesterase.

Resumo

O objetivo deste trabalho foi avaliar, por meio de um método indireto, a presença de substâncias anticolinesterásicas no tabaco de cigarros produzidos no Brasil. Os compostos organofosforados e carbamatos, que são usados nas lavouras

do tabaco, são as principais substâncias com essa característica. Foram analisadas as dez principais marcas de cigarros manufaturados no Brasil. De cada marca de cigarro, foi separado o tabaco e extraídas as substâncias anticolinesterásicas, seguindo um método descrito por Leite (1980) e empregando o n-hexano como solvente. Após o preparo do extrato, foi coletado sangue e separado o plasma para a determinação da atividade da butirilcolinesterase (BChE), empregando-se o método descrito por Dietz et al. (1973). A atividade enzimática foi medida na presença dos extratos de cada uma das marcas, observando-se uma significativa inibição da atividade da BChE com valores médios de 20,16% ($p < 0,001$).

***Palavras-chave**: Organofosforados. Carbamatos. Tabaco. Butirilcolinesterase.*

Introduction

Brazil is the most important exporter country of tobacco leaves and the second world-wide producer, in the southern of the country is located 92.7% of the national cultivated area by families who possess small properties and powerful transnational corporations that industrialize the tobacco (1).

For the production of tobacco derivatives, many toxic substances are used worldwide, such as organophosphate and carbamates compounds, both of them have been used as insecticides. These substances affect the central and peripheral nervous systems, inhibiting a very important enzyme: the erythrocyte acetylcholinesterase EC 3.1.1.7 (AChE-E) (2), in human plasma there is another enzyme: butyrylcholinesterase EC 3.1.1.8 (BChE) (3) also is inhibited by the same substances and have being used as a biological marker for occupational exposure control in many countries including Brazil (4).

The mechanism of action of these compounds is associated with inhibition of brain acetylcholinesterase (AChE-B) in the nervous system. As this enzyme hydrolyzes acetylcholine, a major neurotransmitter in the central, somatic and parasympathetic nervous systems, upon AChE-B inhibition, acetylcholine released from nerve terminals accumulates and over-stimulates muscarinic and nicotinic receptors. The signs and symptoms of organophosphate and carbamate poisoning reflect those of a cholinergic crisis and include central and peripheral nervous system manifestations (5)

The chronic exposition to organophosphate and carbamate compounds is related to neurological toxicity (6, 7), teratogenesis (8), lower sperm concentration (9).

Jin et al. (10) have described a method to detect organophosphate and carbamate substances using the acetylcholinesterase inhibition, the percentage inhibition of enzyme activity is correlated to the pesticide concentration. Nagatani et al. (11) developed enzymatic assay involving AChE to detect organophosphate pesticides in food. Nanda Kumar et al. (12) and Weins and Jork (13) created thin-layer chromatography methods where the separated components were detected by enzyme inhibition.

For organophosphate and carbamate detection in many kind of samples, have been used gas and liquid chromatography methods (14, 15), these methods, unfortunately are too much expensive for many laboratories, so in this paper, we are describing a very simple, fast and inexpensive method to search organophosphate and carbamate compounds in tobacco from Brazilian manufactured cigarettes through inhibition of human BChE

Materials and methods

After a research about important tobacco industries in Brazil, were tested the ten more consumed cigarettes labels in this country. The cigarettes were numerically identified and the anticholinesterasic insecticides were extracted from these samples using the method developed by Leite cited by Moraes (16).

The tobacco from each one of the labels was putted into a 150 mL erlenmeyer with 80 mL of n-hexane and shacked during 30 minutes. After that, the extract was filtered and concentrated in a 45 ºC water bath until complete evaporation of the solvent.

The butyrylcholinesterase (BChE) activity was determined by the Dietz et al. (17) method. At first, female human blood sampled heparinized was used to evaluate the BChE basal level activity. To verify weather n-hexane can interfere in enzyme activity, this activity was evaluated with the solvent (60 μL of n-hexane in 0,6 mL of plasma).

In the Dietz et al. (17) method the BChE hydrolyzes the substrate (propionylthiocholine) to yield free sulfhydryl, which reacts with the color reactive (DTNB – 5,5' – dithiobis (2-nitrobenzoic acid) to yield the 5-thio-2-nitrobenzoate ion, this ion is yellow and has a useful maximum absorption at 410 nm. The Table 1 summarizes the method.

After verify that the solvent cannot interfere in enzyme activity, each one of the extracts were individually tested (60 μL of extract in 0,6 mL of plasma). The percentage inhibition from base line activity was calculated and statically tested through Student's "t" test, the software BioEstat 5.0 developed by Ayres et al. (18) was used for the calculations.

Results

After the BChE basal level and solvent (n-hexane) activities had been evaluated, no difference was observed. BChE basal level activity corresponded 6,710 IU/L and BChE activity in presence of solvent (n-hexane) corresponded 6,740 IU/L.

Table 2 shows the enzymatic activities evaluated in the presence of the tobacco extracts. All the ten tested labels had inhibited, BChE activity when compared to the control (BChE basal level). The average enzymatic inhibition was 20.16% ± 2,14%, this inhibition magnitude is statically significant ($p < 0,001$).

Discussion

The organophosphate compounds are extremely used in tobacco culture currently in Brazil (19). Viana et al. (20) cite many health problems related to chronic exposition to these substances, like neuropathy and depression. Falk et al. (21) had observed a high frequency of suicides in Venâncio Aires, a city located in Rio Grande do Sul, a Brazilian Southern State, this city's economy comes basically from the tobacco, these researchers had raised the hypothesis that this phenomenon would be related with the use of organophosphate compounds in this plantation.

In the cigarette's smoke are found more than 4,700 substances, among them are the organophosphate and carbamate compounds. Mello-da-Silva and Fruchtengarten (22) comment that the children, due the physiological characteristics and habits of their parents, are more exposed to cigarette's smoke with these compounds than adults, these researchers cite the organophosphates malathion, chlorpyriphos and diazinon and the carbamate carbaryl.

We had concluded that this method is very simple, fast and not expensive, being able to be applied for search of anticholinesterasic compounds in many kinds of samples, including tobacco, it will be useful mainly where other methods, as the gas chromatography is not available or cannot be used to control the use of these toxic compounds. The next step it will be the standardization of the method.

Table 1 - Protocol for the procedure

	Tube (mL)	
Reactives	**Blank**	**Test**
Substrate (propionylthiocholine iodide) 45 mmol/L	0,5	0,5
Color reagent (DTNB) 0,423 mmol/L	1,5	1,5
Warm to 37 ºC (5 minutes)		
Serum*	–	0,01
Incubation for 2 min and 30 seconds at 37 ºC		
Inhibitor solution (quinidine sulfate) 0,5%	1,5	1,5
Serum*	0,01	–
Read the absorbance of the test at 410 nm against the corresponding blank within 15 minutes		

Note: * = For basal level activity is used serum without n-hexane or extract.

Table 2 - The BChE activity was lower than base line activity after addition of the extracts ($p < 0,001$), this shows the anti-cholinesterasic effect of the extracts

Reactives	Blank	Test
1	5,690 IU/L	15,20
2	4,630 IU/L	31,00
3	5,980 IU/L	10,88
4	5,510 IU/L	17,88
5	5,250 IU/L	21,76
6	4,980 IU/L	25,78
7	5,070 IU/L	24,44
8	4,950 IU/L	26,23
9	5,540 IU/L	17,44
10	5,970 IU/L	11,03

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