Acute toxicity of ibogaine and noribogaine

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Key words: *ibogaine; noribogaine; median lethal dose; toxicity; mice.*

Summary. *Objective. To evaluate acute toxic effect of ibogaine and noribogaine on the survival of mice and determine median lethal doses of the substances mentioned.*

Material and methods. White laboratory mice were used for the experiments. Ibogaine and noribogaine were administered intragastrically to mice via a stomach tube. Control animals received the same volume of saline. The median lethal dose was calculated with the help of a standard formula.

Results. To determine the median lethal dose of ibogaine, the doses of 100, 300, 400, and 500 mg/kg were administered intragastrically to mice. The survival time of mice after the drug administration was recorded, as well as the number of survived mice in each group. Upon administration of ibogaine at a dose of 500 mg/kg, all mice in this dose group died. Three out of four mice died in the group, which received 300 mg/kg of ibogaine. No mouse deaths were observed in the group, which received 100 mg/kg of ibogaine. The determined LD_{50} value of ibogaine equals to 263 mg/kg of body mass. In order to determine the median lethal dose of noribogaine, the doses of 300, 500, 700, and 900 mg/kg were administered to mice intragastrically. Noribogaine given at a dose of 500 mg/kg had no impact on the mouse survival. The increase of noribogaine dose to 700 mg/kg of mouse body mass led to the death of three out of four mice in the group. Upon administration of noribogaine at a dose of 900 mg/kg, all mice in this group died. The LD_{50} value of noribogaine in mice determined on the basis of the number of dead mice and the size of the doses used equals to 630 mg/kg of mouse body mass. The behavior of mice was observed upon administration of ibogaine or noribogaine. Low doses of ibogaine and noribogaine had no impact on the mouse behavior. External effects (convulsions, nervous behaviour, limb paralysis) were observed only when substances were administrated at higher doses.

Conclusions. It has been determined that the median lethal dose of ibogaine and noribogaine equals to 263 mg and 630 mg/kg of mouse body mass, respectively. The toxicity of ibogaine is 2.4 times higher than that of noribogaine.

Introduction

Looking for new medications for the treatment of drug and alcohol dependence encourages us to focus more attention on and investigate an indole alkaloid ibogaine. There are findings demonstrating its capability to attenuate craving for alcohol (1). However, its toxicity and lethal dose are still unknown. In addition, an active metabolite of ibogaine, noribogaine, has been identified and is currently being analyzed.

Naturally occurring ibogaine is a psychoactive alkaloid extracted from the *Tabernanthe iboga* shrub. For many years, extracts of *Tabernanthe iboga* have been used as central nervous system (CNS) stimulants at low doses or as hallucinogens at high doses (2). Preclinical studies have demonstrated that ibogaine reduces craving for cocaine and morphine, attenuates morphine withdrawal symptoms (3). Based on the clinical studies, a conclusion can be made that ibogaine has a certain antiaddictive action (4). However, its mechanism of action is still not clear enough. The identified antagonistic activity of ibogaine on *N*-methyl-*D*-aspartate receptors as well as its agonist activity on opioid receptors can be regarded as a possible mechanism of antiaddictive action (5). It should be mentioned that ibogaine interacts with several neurotransmitter systems, including serotonin uptake sites and sigma sites. Some of ibogaine actions can be attributed to its long-lasting metabolite, O-desmethyli-

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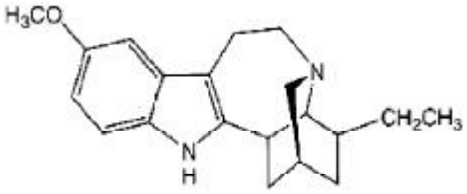
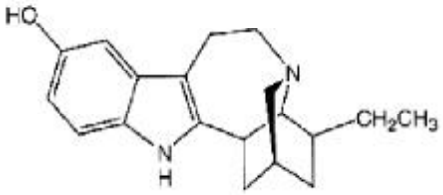


Ibogaine



12-Hydroxyibogamine
(Noribogaine)

Fig. 1. **Structure of ibogaine and noribogaine**

bogaine (other names are noribogaine or 12-hydroxyibogamine) (2). Noribogaine differs from ibogaine in that it contains a hydroxyl instead of a methyl group at position 12 (Fig. 1). Following ibogaine administration, noribogaine has been detected in human plasma (6) as well as in plasma and in the brain of ibogaine-treated rats (7), which proves once more that noribogaine is a metabolite of ibogaine. Experimental studies on rats have established that noribogaine is pharmacologically active and produces effects that mimic those of ibogaine: decrease in craving for morphine and cocaine, reduction in the locomotor effect of morphine (8). Other data presented in literature (9), however, demonstrate that noribogaine produces no positive action in respect of inhibition of the morphine withdrawal signs.

Ibogaine and noribogaine can evoke different behavioral effects despite having similar chemical structures (10, 11). Moreover, it appears that the mechanisms of antiaddictive effects of ibogaine and noribogaine may involve different patterns, which call for more detailed studies. In this connection, our objective was to evaluate acute toxic effect of ibogaine and noribogaine on the survival of mice and to determine median lethal doses (LD_{50}) of these substances.

Materials and methods

Experiments were done on 4–6-week-old outbred mice weighing 20–25 g. All experiments were performed according to the Law on the Care, Keeping and Use of Animals, Republic of Lithuania (License of State Veterinary Service for Working with Laboratory Animals, No. 0153). Before starting experiments, animals were acclimatized to laboratory conditions. Mice were randomly assigned to groups and weighed. Ibogaine and noribogaine are almost completely insoluble in water, so suspensions were used for their administration. Study substances were administered intragastrically to mice via a stomach tube. Control mice received the same amount of saline. The same method of administration was used.

LD_{50} was calculated with the help of the following formula (12):

$$\lg LD_{50} = \lg D_N - \delta(\Sigma L_i - 0.5),$$

where D_N is the highest dose of the study substance administered to mice; δ is the logarithm of the ratio between the doses of the substance administered; L_i is the ratio of the number of dead mice to the number of mice used to determine the dose effect.

Results

Toxicity studies of various drugs and comparison of toxic effects of different substances on the body require evaluation of LD_{50} of such drugs and substances. Determination of the LD_{50} value allows for the correct planning of an experiment not being afraid of overdosing the study drug. Moreover, this value allows for the comparison of the toxicity of various substances. LD_{50} is a calculated single dose of a substance expected to kill 50% of studied animals.

To determine the median lethal dose of ibogaine, we used the following substance concentrations: 500 mg/kg (working suspension concentration of 25 mg/mL), 400 mg/kg (20 mg/mL), 300 mg/kg (15 mg/mL), and 100 mg/kg (5 mg/mL). Each of these doses was administered intragastrically to four mice via a stomach tube. Afterwards, the survival time of mice after the drug administration was recorded, as well as the number of survived mice in each group. Upon administration of the highest ibogaine dose (500 mg/kg), all mice in this group died. No mouse deaths were observed in the last group only, which received the lowest ibogaine dose (100 mg/kg). In the preceding group, which received 300 mg/kg of ibogaine, three mice out of four in that experimental group died (Fig. 2). The LD_{50} of ibogaine was calculated according to the formula specified in the section "Materials and methods." The determined median lethal dose of this drug in mice is 263 mg/kg of body mass (Fig. 3).

To determine the median lethal dose of noribogaine, we used the following substance concentrations:

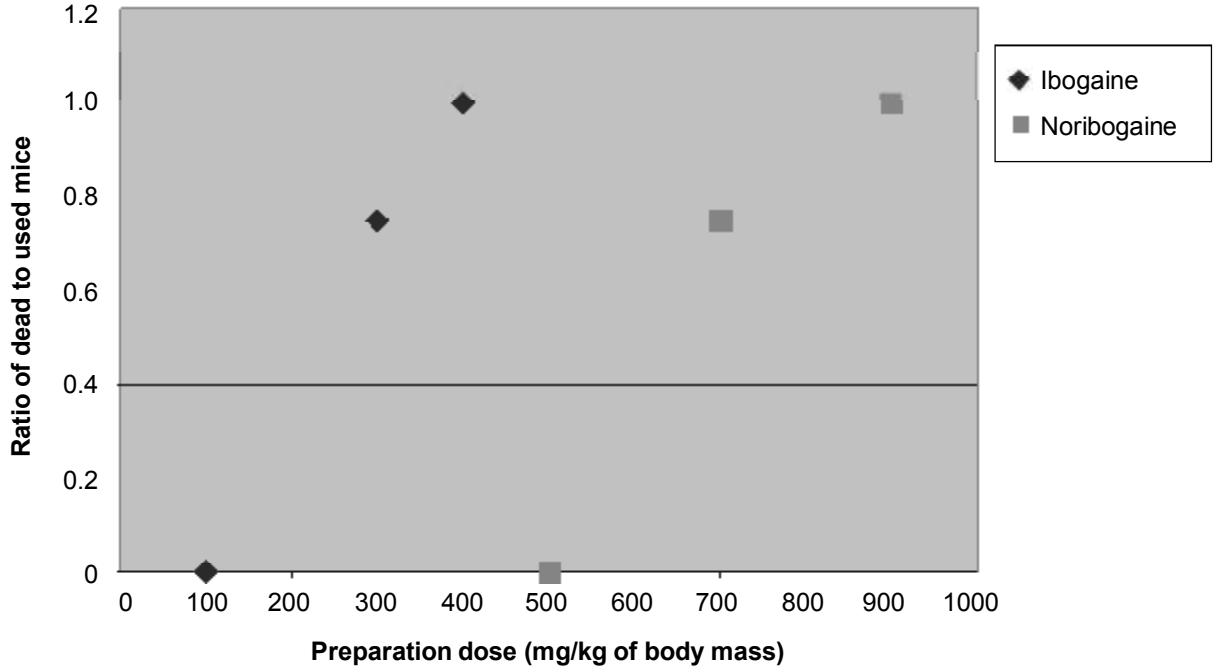


Fig. 2. **The dependence of the mouse survival on the dose of drug administered**
Four mice received the different dose of drug.

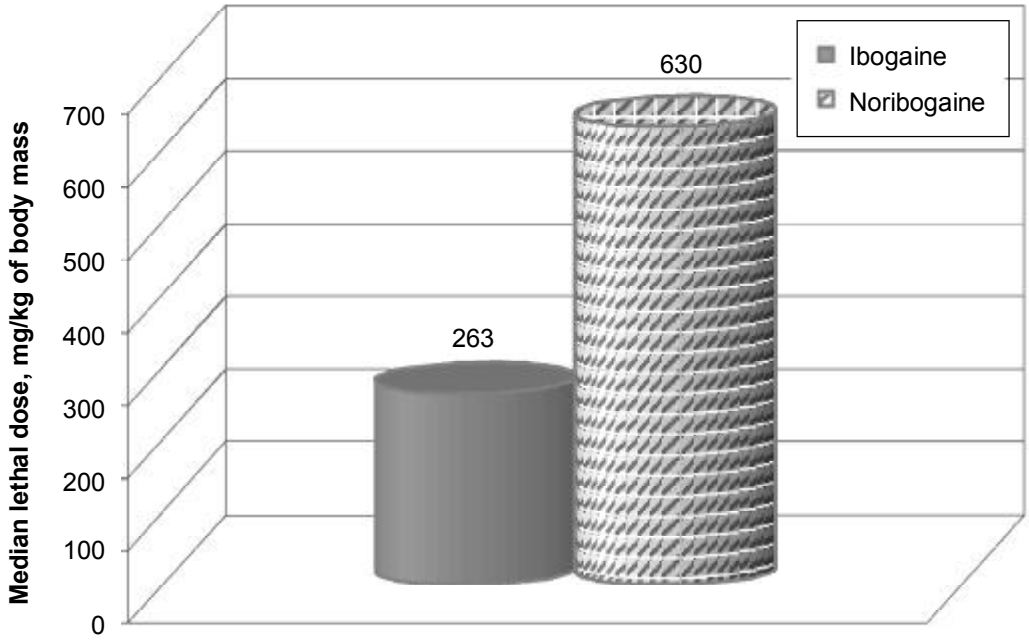


Fig. 3. **Median lethal doses of ibogaine (n=12) and noribogaine (n=16) for mice**

900 mg/kg (working suspension concentration of 45 mg/mL), 700 mg/kg (35 mg/mL), 500 mg/kg (25 mg/mL), and 300 mg/kg (15 mg/mL). The highest dose used in the ibogaine arm of the experiment (500 mg/kg) had no effect on the mouse survival in the noribogaine arm (Fig. 2). That is, all four mice in this experimental group survived. Therefore, we increased the dose of noribogaine to 700 mg/kg of mouse body mass. In this case, three out of four mice in the group died. Following the technique used, the noribogaine dose had to be increased to the higher level (to 900 mg/kg) in order to detect the group, in which all mice die. According to the determined number of dead mice and the doses used, we calculated the LD_{50}

for mice, which is 630 mg/kg of mouse body mass for noribogaine (Fig. 3).

The behaviour of mice was observed following administration of both ibogaine and noribogaine. Behaviour is one of the markers of the substance toxicity in animals. Upon administration of low ibogaine and noribogaine doses, no changes in the behavior of mice were observed. External effects (convulsions, nervous behavior, limb paralysis) of the drugs were observed only in case of administration of higher doses of substances: ibogaine at a dose of 400 mg/kg and noribogaine at a dose of 500 mg/kg of body mass.

Discussion

Data presented in literature show that LD_{50} varies depending on the animal. The route of administration into the body of laboratory animals is also of major importance. The LD_{50} of ibogaine has been determined in guinea pig (82 mg/kg intraperitoneally) and rat (327 mg/kg orally and 145 mg/kg intraperitoneally) (13, 14). No changes in rat liver, kidneys, heart, and brain have been established during the chronic ibogaine toxicity studies (10 mg/kg for 30 days and 40 mg/kg for 12 days) (13). No evidence of neurotoxicity has been found in monkeys given ibogaine at doses of 5–25 mg/kg orally for four consecutive days (15). Other investigations have revealed that ibogaine causes neurotoxic effects, i.e., induces degeneration of Purkinje cells (16) and that the neurotoxicity of ibogaine is dose-dependent (17). Based on the data presented in literature, after intraperitoneal and subcutaneous injection of ibogaine in rats, the highest level of the substance is achieved in brain and adipose tissue one hour after administration. Thus, it can be stated that the potent effect induced by ibogaine in the brain lasts for up to 12 hours following administration, and the further action is determined by active metabolite noribogaine (13). The study by Baumann et al. has demonstrated that in rats, the ratio of noribogaine to ibogaine in the bloodstream is much higher when ibogaine is injected by the intraperitoneal route rather than the intravenous route (11). In our study, we are also planning to study and compare distribution of these substances in internal organs (liver, kidneys, heart, spleen), brain, smooth muscles, and blood when ibogaine and noribogaine are administered directly into the stomach via the stomach tube.

Glick et al. (18) and O'Hearn with Molliver (19) have proved that ibogaine induces tremor and ataxia when administered intraperitoneally at the dose ranging from 40 to 100 mg/kg, meanwhile noribogaine does not cause such effects. In our study, both study substances had an impact on the mouse behavior: ibogaine at a dose of 400 mg/kg and noribogaine at a dose of 500 mg/kg. Since noribogaine shows lower toxicity, it can be more promising for the clinical use.

Conclusions

1. The median lethal dose of both drugs studied was determined. LD_{50} of ibogaine equals to 263 mg/kg and LD_{50} of noribogaine is 630 mg/kg of body mass.
2. The comparison of ibogaine and noribogaine toxicity for mice was performed. It was detected that the latter is 2.4 times lower than that of ibogaine.

Ūminis ibogaino ir noribogaino toksiškumas

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Raktažodžiai: ibogainas, noribogainas, vidutinė mirtina dozė, toksiškumas, pelės.

Santrauka. *Tyrimo tikslas*. Įvertinti ibogaino ir noribogaino toksinį poveikį pelių išgyvenimui bei nustatyti šių medžiagų vidutinę mirtiną dozę.

Tyrimo medžiaga ir metodai. Eksperimentai atlikti su baltomis laboratorinėmis pelėmis. Ibogaino ir noribogaino zondu suleista į pelių skrandį. Kontroliniams gyvūnams skirtas toks pat fiziologinio tirpalo tūris. Vidutinė mirtina dozė apskaičiuota pagal standartinę formulę.

Rezultatai. Vidutinei mirtinai ibogaino dozei nustatyti pelėms skirtos 100, 300, 400 ir 500 mg/kg dozės. Registruotas laikas, kurį išgyveno pelės po preparato suleidimo, ir kiek pelių kiekvienoje grupėje išgyveno. Suleidus 500 mg/kg ibogaino dozę visos grupėje buvusios pelės nugaišo. Grupėje, kur pelėms skirta 300 mg/kg ibogaino dozė, krito trys pelės iš keturių. Skyrus 100 mg/kg ibogaino dozę visos pelės išgyveno. Nustatyta

LD_{50} reikšmė ibogainui lygi 263 mg/kg kūno masės. Vidutinei mirtinai noribogaino dozei nustatyti pelėms per zondą suleistos 300, 500, 700 ir 900 mg/kg dozės. 500 mg/kg noribogaino dozė jokio poveikio pelių išgyvenimui neturėjo. Padidinus noribogaino dozę iki 700 mg/kg pelės kūno masės, krito trys iš keturių grupėje buvusių pelių. Skyrus 900 mg/kg ibogaino, nustatyta, kad šioje grupėje žuvo visos pelės. Pagal žuvusių pelių skaičių ir dozių dydį nustatyta noribogaino LD_{50} reikšmė pelėms lygi 630 mg/kg pelės kūno masės. Suleidus ibogaino ar noribogaino, stebėta pelių elgsena. Mažos ibogaino ir noribogaino dozės pelių elgesio neveikė. Išorinis poveikis (traukuliai, nervingas elgesys, kojų paralyžius) užfiksuotas tik suleidus didesnes medžiagų dozes.

Išvados. Nustatyta, kad vidutinė mirtina dozė yra 263 mg ibogaino ir 630 mg noribogaino kg pelės kūno masės. Ibogainas yra 2,4 karto toksiškesnis už noribogainą.

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