

Nalbuphine: some aspects of the research and applications

Nalbufina – niektóre aspekty badań i zastosowań

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Abstract

Nalbuphine hydrochloride is a synthetic, non-scheduled opioid agonist/antagonist analgesic, widely used across different branches of medicine. Despite the fact that nalbuphine has been used in the clinical setting for more than 40 years, there is still a lot of controversy regarding its mechanism of action and side-effects, including the development of the addiction to the drug. Recent data demonstrated the increase of non-medical use of nalbuphine. Moreover, in some countries it was placed in list of psychotropic and addictive substances. The increasing popularity of nalbuphine led us to review and analyse the data regarding both clinical and non-clinical applications of the drug. Furthermore, we performed an extensive analysis regarding available experimental models and approaches used in the research of opioid substances. Despite a set of problems in clinical settings due to the opioid nature of nalbuphine, it belongs to an indispensable group of analgesics for pain control.

Streszczenie

Chlorowodorek nalbufiny jest syntetycznym, opioidowym agonistycznym i antagonistycznym lekiem przeciwbólowym, szeroko wykorzystywanych w różnych gałęziach medycyny. Mimo że nalbufina jest stosowana w postępowaniu klinicznym od ponad 40 lat nadal istnieje wiele kontrowersji dotyczących mechanizmu jej działania i skutków ubocznych, w tym uzależnienia od narkotyków. Ostatnie dane dowodzą zwiększonego niemedycznego wykorzystania nalbufiny. W niektórych krajach nalbufina została umieszczona w wykazie substancji psychotropowych i uzależniających. Wzrastające zastosowanie tego leku skłania do przeglądu i analizy danych dotyczących klinicznych i nieklinicznych jego aplikacji. Ponadto przeprowadzono analizę dotyczącą dostępnych modeli eksperymentalnych i podejść stosowanych w badaniach substancji opioidowych. Pomimo licznych problemów w praktyce klinicznej ze względu na opioidowy charakter nalbufina należy do grupy analgetyków niezbędnych w kontroli bólu.

Introduction

Nalbuphine – synthetic opioid analgesic, (–)-17-(cyclobutylmethyl)-4,5 α -epoxymorphinan-3,6 α ,14-triol hydrochloride. In chemical structure it is close to morphinan and phenanthrene. Based on its pharmacological action, it belongs to the group of agonist-antagonist opioid receptors (pentazocine, buprenorphine, butorphanol) with agonistic effect on κ receptors and antagonistic effect on μ -receptors, which explains its low impact on the psycho-emotional state of patients compared to morphine, and the virtual absence of addiction in therapeutic doses and short course of usage. In addition, there is a sig-

nificantly lower risk of respiratory complications and complications of the digestive tract, while the analgesic effect is as powerful as that of morphine. High level of analgesic effect and low risk of various complications including saturation effect or “ceiling effect” (reaching a certain threshold and the subsequent lack of effect with an increase in dose) compared to other agonist-antagonists such as pentazocine or buprenorphine [1] have made it the only medication in its pharmacological group, which is the most widely used in the clinical setting today. Its widespread use is also attributed to the fact that it is one of the few opioid drugs that are officially not added to the group of narcotic

and psychotropic drugs. Note also that the question of the saturation effect is ambiguous in experimental works, since from the very beginning of its study it manifested differently in different species, mainly in dogs and rats [2]. The first mention of nalbuphine in literature was in 1978 [3], and it is available for sale in the US. However, any published detailed information about its practical use in medicine appears in the middle of the 1980s [1, 2, 4–7]. Among the side effects observed and described are bradycardia or tachycardia, hypertension or hypotension, a variety of disorders of the central nervous system (depression, dizziness, euphoria, hallucinations etc.), nausea and vomiting, as well as hyperalgesia. In addition, the drug should be prescribed with caution to patients who previously had other opioid analgesia because the presence of properties to block the μ -receptors in nalbuphine can cause withdrawal symptoms. The same applies to drug addicted patients, in which case increased attention to the patient's history is required, and it may create certain difficulties in emergency use. According to the 2014 guidelines for postoperative pain management applicable in Poland [8], nalbuphine is recommended for weak to moderately severe pain. Nalbuphine is not recommended for patients who are addicted to opioids and are being treated chronically with opioids/MOR agonists (withdrawal symptoms, including strong pain, can be substantially exacerbated); moreover, the combination of nalbuphine with other opioids/MOR agonists is not advocated.

Pharmacokinetics and pharmacodynamics. Administration

Maximum concentration is attained in 15–30 min; the effect lasts for 4–6 h; half-life concentration lasts for about 5 h, exiting the body mostly with bile, although a certain amount as metabolites and unchanged drug is found in the urine [2, 9]. The median effective dose (ED₅₀) is 5.4 mg for men and 7.1 mg for women [10], although this figure needs clarification. Maximum single recommended dose: 20 mg; maximum daily recommended dose: 160 mg. Nalbuphine is available in the form of ampoules of 1 ml, 10% or 20% for intramuscular, subcutaneous, and intravenous injection. Oral forms are not used in clinical practice due to the high degree of conjugation in the liver at first pass (first-pass effect). However, the search for an optimal combination for oral administration continues to this day [11], while the emergence of forms with prolonged action opens new possibilities in practical use [12], especially as the authors indicate a half-life of 14.2 h, which is much higher than administration in the form of injections. Like most opioids, metabolism takes place in the liver, primarily, via the cytochrome P-450 (CYP3A4). The second way it occurs is via conjugation with glucuronic acid, which forms inactive metabolites [13]. Conjugation with subsequent intro-

duction through the biliary tract has long been considered the main mode of output for nalbuphine. Of importance is also the phenomenon of secondary adsorption when, after ingress of bile into intestines, the deconjugation takes place involving microorganisms, which leads to re-absorption of the drug. However, in 2014 it was proven that there are at least two hydroxylated forms (3-hydroxynalbuphine, 4-hydroxynalbuphine) and two conjugated (nalbuphine 3- β -d-glucuronide, nalbuphine-6- β -d-glucuronide). The main form is nalbuphine 3- β -d-glucuronide, the concentration of which is about 10 times greater than nalbuphine-6- β -d-glucuronide (when administered orally) [14], but the question about the peculiarities regarding the influence of these forms and their concentration depending on the shape and speed of administration on organs and systems in the oxidative processes, remains open. The same can be said about the activity of conjugated forms and metabolites because the data is practically not available. There are only references to the unpublished data by DuPont Pharmaceuticals, indicating that conjugate is not active and one of the metabolites has high antagonistic properties but a small analgesic effect [2].

Many questions about the mechanisms of action of nalbuphine remain open, in particular the impact on μ -receptors, the influence on the psycho-emotional state, the effect of increasing pain at low doses of medication, and nalbuphine's part in oxidative processes. Some studies have argued nalbuphine's antagonism to μ -receptors, including Preston *et al.* [15], noting the similarity of the antagonistic action of nalbuphine to naloxone in methadone-dependent patients and the lack of μ -agonist effect in this group of patients. It is important to mention nalbuphine's widespread use as a drug used to treat itching [16] after the use of morphine and other narcotic analgesics and for the restoration of breathing arising as a result of using μ -agonists, while the analgesic effect is being retained. These facts indicate antagonism to the μ -receptors. However, many authors noted an effect similar to μ -agonist on psycho-emotional state during clinical use of the drug – Zacny *et al.* [17] demonstrated in a study conducted on volunteers with no experience of drug use history that even small doses (10 mg intravenously) cause a psycho-emotional experience similar to morphine. However, nowadays such action is considered a manifestation of all these effects on the central κ -receptors, although we have not found any experimental confirmation of that. Psychoemotional complications from receiving nalbuphine in drug addicts were also observed and recorded in experimental experience, in particular by Woods and Gmerek [18]. In addition, Jaillon *et al.* [19] pointed out the pharmacodynamic dependence on the age of patients, showing that children metabolise the drug faster than adults, and observed the slowest pharmacokinetics in older people, explaining the difference in blood flow

through the liver. Therefore, special attention is required when administering nalbuphine to children [20] due to accelerated metabolism. In addition, Gear *et al.* [21–23] proved the dependence of the effective dose and, accordingly, the risk of complications on sex. Thus, in women the analgesic effect occurs at much lower doses than in men, which is characteristic of all agonist-antagonists. Overall, the androgen-dependent efficacy of opioids repeatedly became the subject of research both in clinical and laboratory studies [10, 24–26]. However, it is important to note that the androgen status affects the pharmacokinetics and pharmacodynamics of a large number of drugs, not just opioids, the metabolism of which is related to cytochrome due to different CYP3A4 activity in men and women [27].

Nalbuphine and addiction

While considering the dependence of metabolism on the androgen status, it is important to point out the cases of nalbuphine abuse observed in bodybuilders and athletes who have used anabolic steroids [28, 29], in which cases the clinical picture was identical, depending on opioids, while in many cases methadone replacement therapy was successfully used as a method of treatment. However, it should be noted that in addition to the use of anabolic steroids, which was noted in most of the cases, patients consumed large doses of nalbuphine, although within the therapeutic range – an average of 60 mg (ranging from 10 to 100 mg), while some of the patients were addicted to cocaine. Therefore, the authors were very careful about conclusions regarding nalbuphine addiction.

In 2005 the Bulletin of Narcotics published an article by Chung, in which nalbuphine was first mentioned as a narcotic drug [30]. The author analyses the dynamics of cases depending on drugs that do not fall under the category of controlled substances in South Korea. According to the author, 2001 was a critical year for nalbuphine, since the number of recorded cases of nalbuphine addiction reached 1520 compared to 110 in 2000. The addiction was formed quite quickly – on average after 1 week of using the drug. But the overall growth began in 1991 when the drug users started using nalbuphine as an alternative to methamphetamine [31]. Because of this, the government of South Korea added nalbuphine to the list of controlled substances in 2001. This allowed them to reduce the number of nalbuphine-dependent patients dramatically.

In 2006 a study was published about the increased consumption of nalbuphine by the population of Derry town in Northern Ireland [32]. However, the authors did not draw any direct conclusions, as the increased consumption is not a reason to accuse the population of the entire city of being addicted to the drug, but this fact raises many questions. In addition,

a full study, as the authors point out, was not possible because of the lack of legal grounds to examine a large number of people on the subject of drug addiction. Therefore, few people were interviewed. In any case, the authors focused their attention on the need for increased control over the use of the drug, and to monitor its possible abuse. In 2012 Shitov *et al.* published a compilation analysis of 50 cases of non-medical consumption of nalbuphine in the Yaroslavl region of Russia [33]. The authors emphasised that in 2011 the nalbuphine consumption in the region increased sharply after the emergence of problems with access to other drugs among drug users, in particular butorphanol, and it showed a tendency to grow. Patients confidently referred to nalbuphine as a “light drug”, which does not cause addiction. Those that used it observed a feeling of psychological comfort, light-headedness, and a “departure from reality and problems”. Among the withdrawal symptoms were noted a light sense of weakness, apathy, insomnia, irritability, “lump” in the epigastric area, and others. Most patients (90%) combined nalbuphine with Dimedrol (diphenhydramine) or other drugs (tropicamide, naphazoline). The drug was administered intravenously with the dose ranging from 20 mg to 120 mg in 1–3 doses. The authors conducted a urine test in patients, using gas chromatography with mass spectrometric detection to study the concentration of the conjugated and free drug in the urine. The fraction of the unchanged drug was $25.5 \pm 16.6\%$, while no dependency of the level of conjugation of the drug from the level of concentration in the urine was found. Despite certain reservations for usage, the need for careful selection of the dose and schemes of usage, the risk of complications in drug addicts, and the described cases of addiction to the drug, most countries do not list nalbuphine as a narcotic drug. In 2008 the US-based Drug Enforcement Administration (DEA) conducted an analysis in response to a motion to prohibit the sale of Nubain (nalbuphine hydrochloride) produced by Endo Pharmaceuticals (Endo). After the study of the existing data in literature, the agency found no grounds to ban the drug or add it to the list of narcotic drugs [34]. However, the question remains open. In 2014, there was a case of using naloxone to treat psychosis, which emerged after the use of nalbuphine in a patient who was not a drug user [35]. This case was described as an unusual manifestation of side effects. The fact that this is the only case found in literature corroborates its sporadic occurrence. Controversial in this context are the studies pointing to the potential use of nalbuphine as a drug for the treatment of opioid addiction. In particular, Voronkov *et al.* [36], in a study involving heroin-dependent volunteers, found a positive effect of the application of nalbuphine using it at the dose of 0.25 mg/kg intramuscularly with the total course of nalbuphine ranging from 2 weeks to 6 months. Also of a note is the experimental work of Abdel-Ghany

et al. [37], who, in experiments on mice, found nalbuphine's ability to block the development of addiction to tramadol, and they recommended exploration of the use of nalbuphine in the treatment of tramadol-addicted patients. However, to conclude whether it is potentially new treatment approach of addiction involving fundamentally new features, or simply a replacement or supportive-therapy, such as methadone therapy or naltrexone therapy, is currently impossible due to the lack of experimental data. Hasty conclusions about the prospects of the replacement therapy have repeatedly been the basis for the emergence of even larger problems in society [38].

The development towards clinical use of nalbuphine

In 2003 data was published indicating that the analgesic efficiency of nalbuphine is higher when simultaneously administered with low doses of the antagonist naloxone [39], which raised more questions about the mechanism of its effects. Gear *et al.* continued their research in this area and conducted a study of the combined use of low doses of nalbuphine and morphine [40], showing that small doses of morphine potentiate the analgesic effect of nalbuphine. Subsequently, the same authors conducted a study on the impact of nalbuphine-naloxone combination on the brain, using functional magnetic tomography [41] and showing areas of high activity after administration of nalbuphine in the temporal lobe, islands, the thalamus (including pulvinar), the caudate nucleus, and the pons. This study allowed a better understanding of the influence of opioids on the central nervous system in more detail. Therefore, clinical and experimental possibilities of nalbuphine's usage are far from being exhausted. It should be also emphasised that combining opioids with the goal of potentiating the analgesic effect, postponing the emergence of saturation effect ("ceiling effect"), and preventing the effect of the abolition and the reduction of side effects, is virtually axiomatic today in the treatment of patients with chronic pain, including patients with pain of tumoural origin [42–44]. The second promising area of laboratory research today is the search for the agonist-antagonist combination, which would have the maximum analgesic effect without the above-mentioned complications [45], using previously acquired experience of clinical and laboratory research and modern possibilities of pharmaceutical chemistry. Also promising is the research of opioids of exclusive peripheral action, which do not penetrate the blood-brain barrier, including the use of their impact on the gastrointestinal tract [46].

Experiments on animals and experimental models

We were not able to find any complete experimental models that were used to study various aspects

of nalbuphine's action in experiments on animals in open sources. The maximum available information on preclinical study is described in Schmidt *et al.* 1985 [1], since, in fact, the author represents a research laboratory of DuPont Pharmaceuticals, the first manufacturer of the drug. However, most of the data in the article refers to the unpublished data of the company and is very limited. Therefore, it is impossible to use this source as a base for building of a model. To analyse the literature using experimental models for the usage of nalbuphine, we will conduct a brief analysis of the morphine preliminary experimental model as the most complete model in the group of opioids. The morphine model has been used for many decades and there is a lot of experience accumulated that can be used in experimental studies on other opioid drugs.

In general, there are four basic models of using morphine, depending on the type of administration [47]:

1. Induced drinking. Water is substituted by a solution with morphine, thus, animals are forced to take the drug. Often, sucrose is added to mask a bitter taste. This causes some complications in the form of diarrhoea in a prolonged experiment, and raises some questions about the influence of sucrose in the process of drug usage and limits the use of morphological and biochemical studies. This model is usually used in the study of a behaviour and physiological experiments. The concentration of the drug in the solution often varies from 0.1 to 0.4 mg/ml [47, 48]. Much less used is the method of delivering the solution through a gastric probe with an accurately defined dose of the drug administered to each animal [49, 50].
2. Injections. All types are used to administer drugs: subcutaneously, intramuscularly, intravenously, and intraperitoneally. It should, however, be noted that sometimes the authors did not indicate the type of injections they used, making it difficult to compare the results of work with other similar studies because, for example, the analgesic efficacy of morphine, plasma concentration, and median lethal dose (LD50) significantly differ in subcutaneous and intravenous type of administration [51]. There are prolonged models with increasing and without increasing the dose, and models with a single dose. With increasing doses, a variety of ranges were used ranging from 5–20 mg/kg to 80–100 mg/kg. Sometimes, the dose reached 320 mg/kg [52]. Stages of increase vary too: 4–8–12 mg/kg/day, increasing every 10 days (histological and biochemical study) [53]; 4–8–10 mg/kg/day with increasing dose every 10 days (biochemical and histological study, with comparisons that were conducted with the control group and the group of tramadol with the dose of 20–40–80 mg/kg/day with an increase every 10 days) [54], 10–20–40 mg/kg/day with an increase every 2 days, the dose of 40 mg remained until 7–21 days (biochemical study) [55]; 20 mg/kg/day 1–5 days,

- 40 mg/kg/day 6–10 days, 60 mg/kg per day 11–20 days (biochemical) [56]; 10–20–40–60–80–100 mg/kg twice a day with a daily dose increase to 7 days (biochemical) [57]; and 20 mg/kg/day 1–2 days, 30 mg/kg/day 3–5 days (rat, biochemical and histological study) [58]. In the case of a single dose they used a dose of 10 mg/kg to 40 mg/kg [59] and 6 mg/kg [60].
3. Self-administration. Mainly used in studies of a drug addiction [61, 62] and in behavioural experiments.
 4. The model of an embedded subcutaneous capsule containing a drug [63]. This model is often used in the drug research too, especially in experiments of self-administration with a drug on the background of morphine sensitisation. An osmotic pump can be considered one of the variations in this model [64].

The first broad overview of issues related to a morphine model was made in 1984 by Flecknell [51] highlighting a high variability in dosage, types of administration, duration of the experiment in different authors, species-dependent physiological effects of a drug addiction, and dependence on the methods used in the experiment. Thus, as was mentioned above, the morphine model is the most elaborated and structured, but even so, many questions remain regarding each individual method of administration and on the choice of the dose selected for a particular study. For example, even such a well-researched method in the study of addiction as self-administration is a subject of debate regarding the objectivity of the methodology and the need for it to be further improved [62]. When reviewing sources with experimental research using nalbuphine, first of all, it should be noted that at present there is neither systematisation of techniques of nalbuphine administration in experimental studies, nor a general point of view on a range of doses and regimens. In addition, the vast amount of experimental works using nalbuphine have concerned the physiological and behavioural responses.

Schmidt *et al.* 1985 – 1.24–124 mg/kg s.c. (more precisely, the results of these authors indicated only doses of 1.24 mg/kg, 12 mg/kg, and 124 mg/kg) for the study of respiratory depression in rats (although the authors justify the dose based on the range of 1–100× from analgesic ED₅₀ in mice, determined based on PQW test); 0.1–100 mg/kg s.c. in rats to determine other side effects; 2.4 mg/kg to 50–200 mg/kg s.c. in dogs to determine side effects in a small, lethal and sub-lethal doses; and 15 mg/kg subcutaneously in monkeys to study behavioural depression. In addition, the authors determined LD₅₀ for mice and rats – 1250 mg/kg s.c., and for dogs – 200 mg/kg s.c. (it should be noted that in addition to the above mentioned doses, the authors do not specify any details about the methods of the research and, as was mentioned above, directly representing DuPont Pharmaceuticals, refer to the “unpublished data” [1]). The analgesic activity defined in the PQW test in mice ED₅₀ = 1.2 mg/kg

s.c.; 7.6 mg/kg p.o., antagonistic activity to drugs (narcotic antagonist) defined in mice through anti-Straub tail test (AST, narcotic antagonism) ED₅₀ = 0.68 mg/kg s.c.; 9.0 mg/kg p.o., antagonistic index – the ratio of analgesic and antagonistic activity PQW ED₅₀/AST ED₅₀ = 1.8 and is close to that of nalorphine. In a rat the PQW test ED₅₀ = 1.4 mg/kg s.c.; 5.4 mg/kg p.o. Antagonist activity to narcotics defined through anticatalepsy test ED₅₀ = 1.8 mg/kg s.c. Errick 1983 [2] also makes references to the unpublished data by DuPont Pharmaceuticals, indicating the following doses: LD₅₀ for mice 490 mg/kg for intravenous administration and 860 mg/kg for oral administration; for rats 182–218 mg/kg for intravenous administration and 1000 mg/kg for oral administration; and for dogs 140 mg/kg for intravenous and 1130 mg/kg for oral administration. Death occurred as the result of tonic-clonic seizures or due to respiratory arrest. Toxicity in prolonged experiment was studied in intravenous or subcutaneous administration of the drug at a dose of 6.6–100 mg/kg/day in rats and 2–50 mg/kg/day in dogs for the duration of 2 weeks to 6 months. Researchers indicate renewable hair loss as the only side effect. The study of the influence on reproductive function and embryotoxicity involved the doses of 14–56 mg/kg/day subcutaneously in rats with no side effects related to the reproductive function identified, 100 mg/kg/day subcutaneously in rats, and 4–32 mg/kg/day intravenously in rabbits with no manifestation of embryotoxicity or dysmorphogenesis found.

DiFazio *et al.* [65], the only author we could find, who used the model with increasing intravenous administration of nalbuphine in the range of 10–200 mg/kg/min also using a single injection in doses of 40 mg/kg intravenously and infusion of 100 mg/kg/min to determine the effect on the level of partial pressure of carbon dioxide (pCO₂) on an analgesic activity. Moreover, the author makes direct comparison and conclusions based on the concentration of nalbuphine in the blood of a rat and a man regarding the impact on the level of pCO₂ as well as the analgesic activity of the drug, setting apart only the indicator of the period of half-excretion of the drug.

Walker and Young [66] and Young *et al.* [67] conducted series of experiments on the ability of animals to distinguish drugs from control (saline) in different combinations, using morphine, etorphine, methadone, dezocine, pentazocine, nalorphine, naltrexone, and nalbuphine, as well as non-opioid drugs: ketamine, amphetamine, and pentobarbital. As the authors stated, “in each interaction study, doses were determined empirically” depending on the response of the animals. For nalbuphine the dose range was determined from 0.1 mg/kg to 32 mg/kg s.c. The optimal dose for further use as a training dose in physiological studies with discriminatory stimulation, according to the authors, was 3.2 mg/kg s.c. In addition, the au-

thors conclude that nalbuphine demonstrates itself as a μ -agonist with low activity in certain reactions.

Guzmán *et al.* [68] conducted a study on the impact of nalbuphine and marijuana extract on processes of peroxide oxidation of lipids and the level of 5-hydroxyindoleacetic acid in the brain of a rat. Nalbuphine was used at the dose of 10 mg/kg. The authors pointed out growth indicators of oxidative stress in the rat's brain tissues as the result of nalbuphine administration.

Abdel-Ghany *et al.* [37] used nalbuphine at the dose of 7 mg/kg s.c. in experiments on mice, combining it with tramadol at the dose of 70 mg/kg s.c. and discovered a potential property of nalbuphine to block the development of addiction to tramadol.

Altarifi *et al.* 2012 [69], 2013 [70], 2015 [71] used nalbuphine in experimental works studying the effects on opioid receptors and the efficiency of μ -agonists and antagonists, using intracranial self-stimulation in rats (ICSS). The range of doses of nalbuphine amounted to 0.032–10 mg/kg (in recent works 0.1–10 mg/kg), while the authors noted a different reaction to nalbuphine in the rats that previously had opioids administered and the rats without previous opioid experience. Based on the analysis of the data, the authors found the reasonable dose of nalbuphine for further study of intracranial self-stimulation to be 1.0 mg/kg, as the most balanced in impact on opioid receptors.

Woods and Gmerek used nalbuphine at the dose of 32 mg/kg every 6 h over 31 days to study the psychoemotional complications from taking the drug in monkeys [18].

Yoo *et al.*, elaborating on the model of the definition of nalbuphine and its metabolites in the urine of drug-addicted patients, conducted experiments on rats using nalbuphine at the dose of 10 mg/kg, examining the rate of excretion of the drug [31]. Smith *et al.* used morphine, levorphanol, buprenorphine, butorphanol, and nalbuphine in experiments on rats, studying the influence of the surrounding environment and social environment on the sensitivity of the opioid system. The range of doses for each drug was determined by cumulative procedures using tail withdrawal test. For nalbuphine the range of doses was defined from 0.3 mg/kg to 30 mg/kg [72].

Onysko *et al.* [73] proposed a 6-week model of physical opioid dependence introducing nalbuphine to rats daily with the defined increase of the dose every 7 days in a sequence of 8 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, and 35 mg/kg. The authors are the only researchers found in the literature by us, who worked to determine the therapeutic range in a rat, actually having modelled the preclinical research of the drug, while formerly defining the maximal lethal dose (LD₀), which, according to these authors, was 700 mg/kg, and then determined the therapeutic range of 5% effective dose (ED₅) = $700/16 \times 0.05 = 2.19$ mg/kg and

90% effective dose (ED₉₀) = $700/16 \times 0.9 = 39.38$ mg/kg. Then they set the above-mentioned sequence.

Note that the therapeutic range for a rat determined by the authors using the formula based on the LD₀ actually matches the ranges in physiological experiments on rats indicated in other sources [66, 67, 69–72] and, therefore, can be considered one of the most valid. In addition, the 7-day interval in collection of material is the most appropriate to study the impact of nalbuphine in possible non-medical use, because such a given term is indicated as minimal for the formation of dependency on nalbuphine [30], while the medical use mostly continues for seven days. Recently this model has been used for several studies of morphological changes in the organs of the rat being under the influence of nalbuphine, including eye [74], pancreas [75], white matter of the brain [76], liver [77, 78], biochemical parameters [79], heart [80, 81], and oral cavity [82].

We proposed a model to study the effects on oxidative processes using a daily dose of 0.9 mg/kg [83]. When selecting doses, we were guided by the following criteria – the dose should be in the range of 0.32–3.2 mg/kg, which maximally covers the indicators and the ED₅₀ for rats as specified by the manufacturer and optimal doses in physiological experiments. We were avoiding large doses because of the possibility of the scavenger effect related to hydrogen peroxide (binding of the hydrogen peroxide by a molecule of the drug), which can be predicted and is inherent to nalbuphine, similarly to morphine [55, 84], since this effect is due to the presence of free hydroxyl groups in the structure of some opioids, and the nalbuphine molecule contains three such groups. The high concentration of the drug could theoretically lead to a significant scavenger effect that could significantly affect the markers of oxidative processes. Aside from that, recalculation of the value per 70 kg of body weight leads us to the dose of 60 mg, which was listed as the average dose in drug users [28, 29, 33] and as a maximum daily dose with practical use in a clinical setting (three injections of 20 mg). It was suggested that the sample materials be collected every seven days with a total duration of up to 42 days.

Conclusions and discussion

The question of practical use of drugs, performance, and possible complications, for which there is not enough evidence, is not scientific. Therefore, we will avoid discussions in that area, since such an approach to opioid drugs often leads to tragic consequences [38]. The task and goal of scientists is to obtain the maximum information about all aspects of the drug. Therefore, the choice of model for experiment is key at the planning stage and is critical to the value and validity of further obtained results. Having analysed the existing information about nalbuphine and experience in

experimental studies of its use, it becomes clear that the choice of a dose for the experiment is not a simple task. The issue is not only about the problem of interpretation of the results obtained in animals for the use in human, but also because the data obtained in experiments on animals varies depending on the methodology of the study. For example, the ED50 for mice is indicated as 1.2 mg/kg s.c. [1] and 41.8 mg/kg s.c. [85]. We believe that, based on experimental studies, the range of doses for rats of maximum 0.1 mg/kg to 30 mg/kg daily (prolonged experiment) is more sound. It is exactly in this range that the most significant behavioural reactions, analgesic activity, and reactivity of opioid receptors are observed. Therefore, this range can be considered as a reasonable therapeutic range for rats. As it was already mentioned, it actually coincides with the therapeutic range, calculated on the basis of established ED0 from 2.19 mg/kg to 39.38 mg/kg [73], although extreme values require correction in accordance with the information presented above. Conventionally, the entire range, based on the literature, can be divided into sub-ranges: 0.1–0.32 mg/kg as minimal dose, 0.32–3.2 mg/kg as the medium dose, 3.2–10 mg/kg as large, and 10–30 mg/kg and more as very large. The choice of sub-bands depends on concrete goals. The next group of important issues, aside from dose, is periodicity, path, and the use of a fixed dose or increasing dose. In particular, as was indicated, both physiological experiments and morphological studies used the dynamic model with increasing dose, and the model with the fixed dose. In many studies the authors pointed out the criteria for choosing a particular method, and by the analysis of the results one can appreciate the advantages or disadvantages that they received as a result of using a specific methodology. Combined with the clinical experience of use of nalbuphine, it facilitates the construction of a model of experimental study depending on the set goal.

Conflict of interest

The authors declare no conflict of interest.

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