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The enzymes involved in the metabolism of cocaine: A new pharmacological approach for the treatment of cocaine overdose toxicity

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Review article

ABSTRACT

Introduction
New therapeutic strategies against cocaine overdose toxicity have been developed. These new approaches are based on the design and synthesis of proteins involved in the destruction of cocaine before it has a chance to penetrate nerve tissue.

Objective
To review the progress in the effect of the increase in the catalytic activity of BChE and hCE enzymes produced for the treatment of patients in cocaine overdose toxicity conditions in order to determine the advantages and disadvantages of its use. Its potential future use in patients channeled by a cocaine overdose is also explored.

Method
A bibliographic search was conducted using PubMed; descriptors were "cocaine", "hydrolase", "esterase" and "butyrylcholinesterase". 220 papers were obtained and 126 papers were used for these review.

Results
The BChE, COCH and Coce bacterial enzymes significantly decrease the levels of cocaine in blood and brain and thereby attenuate the effects of a cocaine overdose.

Discussion and conclusion
The results obtained in animal models suggest the potential therapeutic use of these enzymes in humans to rapidly inactivate cocaine and develop treatments to stop deaths associated with cocaine overdose intoxication. These enzymatic approaches offer a novel therapeutic application to treat cocaine overdose.

Key words: Addiction, cocaine, enzymes, and pharmacotherapy.

RESUMEN

Introducción
Se han desarrollado nuevas estrategias terapéuticas contra la toxicidad por sobredosis de cocaína basadas en el aumento en la actividad catalítica de enzimas que participan en la destrucción de su molécula, antes de que tenga la oportunidad de penetrar el tejido nervioso.

Objetivo
Describir los avances en el efecto del aumento en la actividad catalítica de las enzimas BChE y las hCE, producidas para el tratamiento de pacientes en condiciones de toxicidad por sobredosis de cocaína, así como mencionar sus ventajas y desventajas y su potencial uso futuro en pacientes internados por una sobredosis de cocaína.

Método
Se realizó una búsqueda bibliográfica por medio del PubMed, usando como descriptores las palabras "*Cocaine*", "*hydrolase*", "*esterase*" y "*butyrylcholinesterase*". Se obtuvieron 220 artículos de los cuales se usaron 126 para esta revisión.

Resultados
Las enzimas BChE, COCH y CoCe bacteriana disminuyeron significativamente los niveles de cocaína en la sangre y el cerebro y con ello atenuaron los efectos de una sobredosis de cocaína.

Discusión y conclusión
Los resultados obtenidos en modelos animales sugieren el potencial terapéutico del uso de estas enzimas en humanos, para inactivar rápidamente a la cocaína y desarrollar tratamientos para evitar las muertes asociadas con la intoxicación por sobredosis.
Estas metodologías enzimáticas ofrecen una aplicación terapéutica novedosa para el tratamiento de la sobredosis.

Palabras clave: Adicciones, cocaína, enzimas, terapia farmacológica.

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INTRODUCTION

Pharmacopeia historically used to attenuate and/or abolish dependency on illegal drugs of abuse with high addictive potential such as cocaine have shown limited therapeutic efficacy in both the short and long term.[1,2] Because of this, for more than a decade various researchers have been developing new therapeutic strategies against addictive drugs such as cocaine.[3,4]

Some research groups have developed pharmacological therapies through the use of new drugs,[5,6] others have validated immunotherapy methods based on active and passive vaccination procedures,[7,8] and still others have explored the use of proteins that involve destroying cocaine molecules before they have the chance to pass through the blood-brain barrier and penetrate the nervous tissue (figure 1-B), such as the increase in catalytic activity of enzymes such as butyrylcholinesterase (BChE)[9-11] and hepatic carboxylesterases (hCE-1 and hCE-2).

Various epidemiological studies have reported that a high percentage of deaths associated with cocaine abuse are generally related to intoxication by overdose, primarily due to a lack of effective therapy.[12] For several years, various research groups have carried out studies aimed at developing and validating certain therapeutic strategies, with relative success. As mentioned previously, one of these strategies has been to increase the catalytic activity of enzymes which metabolize the cocaine molecule. Various studies have been reported which describe how the activity of these enzymes has been maximized through molecular biology techniques; other studies have described the effect of treatment with these enzymes on rodents and humans. However, there has not been a review that describes the benefits, advantages, disadvantages, and potential future uses of an increased catalytic activity of the enzymes which metabolize cocaine, BChE, and hCs. The aim of this review was to analyze the scientific advances related to an increase in the catalytic activity of the BChE and hCE enzymes, with the aim of describing their main biological effects and possible future use in treating patients in conditions of toxicity due to cocaine overdose.

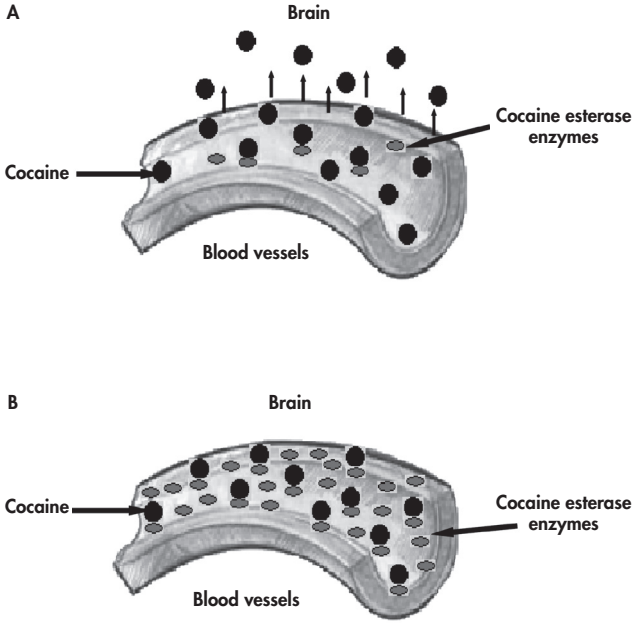

Figure 1. A) Cocaine rapidly moves into the blood vessels, crosses the blood-brain barrier, and reaches its target site within the brain. Enzymes which hydrolyze cocaine (BChE, CocE) are located within the blood vessels, but they are few, and their hydrolytic capacity is limited, so they are not very efficient. (B) When pure or genetically-modified enzymes are administered (with increased hydrolysis capacity and half-life) they rapidly capture cocaine within the blood vessels, hydrolyze it into its inactive metabolites, and impede its reinforcing or toxic effects.

METHOD

A bibliographic search was carried out using the PubMed search engine with the following search terms: Cocaine, butyrylcholinesterase, hydrolase, and esterase. The search was carried out covering a period from January 1970 through December 2015. The algorithm for the search was: ("cocaine"[MeSH Terms] OR "cocaine"[All Fields]) AND ("hydrolases"[MeSH Terms] OR "hydrolases"[All Fields] OR "hydrolase"[All Fields]) AND ("esterases"[MeSH Terms] OR "esterases"[All Fields] OR "esterase"[All Fields]) AND ("cholinesterases"[MeSH Terms] OR "cholinesterases"[All Fields] OR "butyrylcholinesterase"[All Fields] OR "butyrylcholinesterase"[MeSH Terms]).

The inclusion criteria were the following: 1) Studies published in indexed international publications, 2) basic, preclinical, clinical, and review research articles that 3) described the structure, biochemistry, and kinetics of the BChE and hCE enzymes, as well as the characterization of the biological-therapeutic effect and biological safety 4) in animals (rodents, rabbits, and primates), and human adults, 5) studies that were carried out in the U.S., Canada, and the European Community, and 6) were published in English, French, or Spanish.

Exclusion criteria were the following: articles which were 1) editorials, expert opinions, or communications to conferences, 2) articles that did not include information relevant to the aim of the study in their content, 3) content that was repeated in the content of another article.

An analysis of the results indicated that the bibliographical search gleaned a total of 220 articles, 126 of which were considered for inclusion in this review. Of these 126, 97 were research articles, nine were clinical research, one was a meta-analysis, and 19 were review articles (figure 2).

RESULTS

Butyrylcholinesterase

Once ingested, cocaine is almost totally metabolized. The main route of transformation is enzymatic hydrolysis, and plasmatic (BChE) and hepatic (hCE-1) esterases are the main enzymes responsible for forming its metabolites: ecgonine methyl ester, ecgonine, and benzoylecgonine (figure 1A).

BChE is the main enzyme that metabolizes cocaine in plasma in both humans and other species.[13-16]

The half-life of BChE in animal plasma is approximately 21.6 hours[17-19] and it quickly metabolizes the cocaine molecule[20-23] into the metabolite ecgonine methyl ester. Hepatic enzymes transform cocaine into the metabolites norcocaine and benzoylecgonine.[20,24-28] This change in the metabolic profile of cocaine has important physiological implications. Some studies have shown that benzoylecgonine is a potent vaso-constrictor[29,30] and causes convulsive crises,[31] and norcocaine is a highly hepatotoxic metabolite and powerful local anasthetic.[32,33] Conversely, methylecgonine ester does not generate any adverse physiological effects and is quickly eliminated by the kidneys, due to which the increase in concentration of this metabolite does not cause toxic effects in the subject.[34]

A variety of clinical evidence suggests that BChE endogen activity is inversely correlated with the severity of toxicity that cocaine can cause in humans.[35,36] Normal levels of BChE vary between individuals and are dependent on age, state of health, exposure to environmental toxins, and genetic factors.[37-39]

Some clinical reports indicate that individuals who suffer severe medical problems after using cocaine tend to show less activity in plasmatic BChE than those who experience less severe problems.[40-42] Furthermore, some genetic studies have reported that in extreme cases of cocaine intoxication, homozygous patients can show a "silent" variant of BChE, which does not express detectable catalytic activity,[43,44] low levels of BChE expression, or even defective or "atypical" variants of the enzyme. These patients experience prolonged responses to cocaine. *In vitro* studies demonstrated that BChE which comes from serums in atypical patients showed a 50% reduction in capacity to hydrolyze cocaine in plasma,[45,46] which upholds the important role played by BChE in the serum of subjects dependent on the drug.

Some pioneering studies have reported that patients dependent on cocaine who have received purified human BChE (obtained from donor serum) have not had adverse clinical events for up to two days,[47,48] which would suggest that administration of BChE could be a useful therapy to treat patients dependent on cocaine.

In animal models, daily administration of cocaine for seven days (20 mg/kg ip.), to BChE *knockout* mice which expressed low or no activity in catalyzing it, quickly caused cardiomyopathy, respiratory depression (for approximately

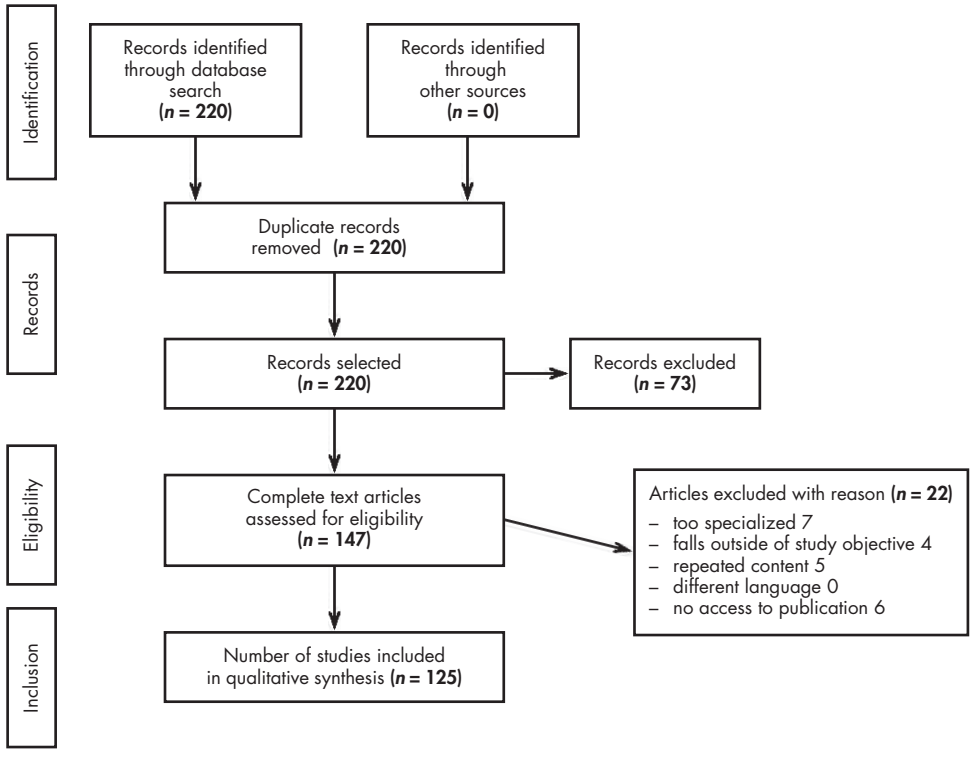

Figure 2. Flow diagram of the study's selection process.

12 hours), abnormal breathing patterns (apneusis), and at a histological level, significant liver toxicity and cardiac perivascular fibrosis.[48] Conversely, mice with normal expression of BChE recovered respiratory rhythm to normal levels 30 minutes after dosing and showed neither apneusis or liver toxicity.[49-51]

The development of a double-mutant mouse has recently been reported, which showed a nil expression of carboxyl-esterase and BChE. When a lethal dose of cocaine was administered (100 mg/kg), the double *knockout* mice showed an increase in the duration of toxic signs (hypothermia, hyperactivity, stereotyped behaviors, ocular effects, and tail dorsiflexion) that was 2.5 times the duration showed by the naive BChE mouse.[50]

Various assessments have reported that administration of BChE (15,000 or 5,000 IU, iv.) derived from horse serum, reduced the half-life of cocaine by 26.2 minutes to 16.4 minutes in the plasma of rodents, cats, and primates.[26,52,53] Furthermore, *in vitro*, rodent, primate, and human BChE also increased the metabolism of cocaine.[54-56]

In terms of cocaine levels in the brain, administration of BChE 7.8 mg/kg, iv.) to rats reduced the concentration of cocaine to 80% in four minutes, 30% at 45 minutes, and 24% at 52 minutes after the administration of cocaine (30 mg/kg, ip.).[26,57-59]

It has been reported that intravenous administration to rats of 5,000 IU of BChE derived from horse serum, followed by intraperitoneal administration of 17 mg/kg of cocaine produced a significant attenuation in the locomotive activity induced by its administration, in sessions of 120 minutes.[56,60] It also temporarily reduced re-establishment of self-administration.[61-64]

In rodents and primates, acute toxicity induced by cocaine overdose was marked by an increase in blood pressure, a reduction in cardiac rhythm, hypertension, bradycardia, respiratory suppression, and tonic-clonic convulsions, the latter being associated with epileptic crises. These are the primary mechanisms responsible for fatality induced by cocaine overdose.[65-67]

In rats, the administration of a 7.8 mg/kg, iv. dose of BChE increased plasmatic levels of the enzyme by more than 800 times the normal level, which avoided hypertension and cardiac arrhythmias caused by cocaine overdose (Lynch 1997). Higher doses in mice (13.7 or 27.4 mg/kg) reduced the incidence of convulsive crises and death produced by doses of up to 80 mg/kg, ip.[68]

However, despite its strategic availability in circulation, the catalytic efficiency of human BChE is very low and depends on many factors. In situations of acute exposure to toxic concentrations of cocaine, BChE is easily overwhelmed.[69,70]

With the aim of increasing the catalytic capacity of human BChE, various research groups carried out successive mutations to hBChE.[71-73] Upon introducing a simple mutation, alanine 328-tyrosine, to transfective ovarian hamster cells, some research groups managed to increase the speed of hydrolysis of cocaine by a factor of 4.[74] If the mutation was tyrosine 332-alanine, the reaction speed increased 40 times. In rats, administration of the mutant BChE blocked convulsive crises and fatality induced by cocaine overdose (100 mg/kg, ip).[75]

Cocaine hydrolase

Later studies with computerized molecular design and genetic engineering[76-80] generated various enzymes capable of hydrolyzing cocaine from human BChE, and these were called cocaine hydrolases (hCocE). A double mutant called "hCocH" was then designed, as well as a quadruple mutant, "AME-359",[81,82] and recently, a hBChE with five simultaneous mutations, called "hCocH2".[83]

In vitro, the "hCocE" hydrolase (A328W/Y332A-BChE) was capable of increasing catalytic efficiency showed by BChE by 1,500 times.[84-86] However, despite the increase in the efficiency of cocaine hydrolysis, the enzyme was not capable of hydrolyzing acetylcholine.

When hCocE (3 mg/kg iv.) was administered to rats, it was capable of quickly removing cocaine from blood vessels, reducing the half-life of the drug from 52 to 18 minutes, reducing the concentration of cocaine in plasma, and thereby reducing its accumulation in the CNS, and it also increased plasmatic levels of benzoic acid, a non-toxic product of cocaine hydrolysis.[87,88]

In vivo, hCocE reduced locomotive activity and attenuated the cardiovascular response (blood pressure) induced by the drug.[89-93]

In studies on cocaine overdose in rats, hCocE has shown better catalytic efficiency and selectivity compared to hBChE. hCocE efficiently blocked the cardiovascular and neurological effects induced by lethal doses (180 mg/kg ip.) in rats and primates.[65] Furthermore, 1 mg/kg of hCocE protected 100% of the animals which received toxic doses of cocaine (180 mg/kg), whereas administration of 13 mg of BChE failed to protect rats from fatality caused by similar doses. hCocE given to rats after the appearance of convulsive crises did not only shorten the duration of these, but also saved the subject from death.[94]

However, despite these results, a significant disadvantage of hCocE is that it has a very short half-life (< 10 minutes) in plasma, which does not allow it to have a long-term protective action.

Bacterial cocaine esterase

The bacteria rhodococcus sp., MB1, is capable of producing an esterase, bCocE, which can hydrolyze cocaine both *in vitro* and *in vivo*.[95] The enzymatic action of this esterase managed to increase up to 1000 times more compared to that

shown by human hBChE, which is 105-106 times faster than a monoclonal antibody.[96]

Administration of bCocE attenuated the re-establishment of drug-seeking behavior in animals previously trained to self-administer cocaine, and it blocked the increase in locomotive activity induced by the same.[97]

Furthermore, bCocE at doses of 28 mg/kg quickly restored blood pressure (three minutes) and hypertension, reduced cardiac arrhythmia, and reduced toxicity induced by overdose (100 mg/kg, 1p.), preventing death by convulsive crises in both rats and mice.[98]

However, despite their efficiency, mammalian enzymes are more effective *in vivo* than bacterial ones. Bacterial cocaine esterase injected into rats had a half-life of only 15 minutes compared with eight hours for human CocH-albumin.[66]

There are many factors that intervene in the length of bCocE's half-life, but the most relevant are the immune response generated by the host against the enzyme, and temperature. Brim et al. reported that process of eliminating bacterial bCocE was dependent on temperature (thermolabile). bCocE has a mean half-life of just 11 minutes at 37°C.[99]

Ko et al. demonstrated that despite bCocE being a very large bacterial protein, due to which it is likely to be able to generate a potent immune response, it withholds its effectiveness after one or more exposures, which suggests that CocE is a weak antigen, not capable of generating a robust immune response.[100] This would suggest that human endogenous temperature is the main obstacle to its use as an effective therapeutic agent.

Mutant esterases

Given that bacterial esterase is unstable at physiological temperatures, various research groups carried out a series of mutations aimed at improving the protein's stability at different temperatures. These mutants, called T172R, G173Q, and L196K, showed significant stability *in vitro* at 37°C. When assessed *in vivo*, the mutant T172R showed a half-life of 78 minutes, while the mutants G173Q and L196K had a half-life at 37°C of 75 and 403 minutes respectively. In terms of hydrolytic activity, the mutant G173Q did not show any alteration in its catalytic activity; whereas the mutant T172R and the double mutant T172R-G173Q showed an increase of three times in their capability to hydrolyze cocaine. Furthermore, the mutant L196K showed an increase of eight times in its catalytic efficiency.[101,102]

In parallel, Gao et al. aimed to increase the hydrolytic activity of BChE, and generated a mutant called AME-359.[103] This enzyme showed an impressive capacity to hydrolyze cocaine in plasma.[104,105] Its catalytic efficiency increased 100 times more than the catalytic activity shown by native human BChE, and it was 450 times higher than that reported for CocE and bCocE.[66,98]

When AME-359 was administered in doses of 0.5 mg/kg, it reduced cardiovascular toxicity induced by a cocaine overdose more efficiently compared to treatment with 3 mg/kg of CocE.[106]

The production of mutants of human BChE in transgenic plants (*Nicotinia benthamiana*) has recently been described. The first mutant developed using this approach was a double mutant of BChE, A328W/Y332A. This showed a significant increase in hydrolytic activity against cocaine.[107]

The catalytic properties of this mutant (called variant 1) were subsequently improved by introducing additional mutants in different parts of human BHcE in order to create the so-called: variant 2 (F227A/S287G/A328W/Y332A), variant 3 (A199S/S287G/A328W/Y332G), 4 (A199S/F227A/S287G/A328W/Y332G) and 5 (F227A/S287G/A328W/Y332G). Variant 4 of human BChE was the most efficient at hydrolyzing cocaine.[107,108]

Hou et al. recently assessed the catalytic capacity of two mutants of human BChE, E14-3 and E12-7, to hydrolyze cocaethylene, a toxic product of cocaine. *In vitro*, enzyme E12-7 improved the catalytic efficiency of human BChE up to 817 times; *in vivo*, E12-7 was capable of efficiently hydrolyzing cocaethylene, cocaine, and norcocaine in rats.[109]

Gene therapy

Other research groups developed and validated other genomic transfer protocols, where the human CocH gene was transferred to a host, by means of an adenoviral vector, with the aim of generating high and sustained plasma levels of cocaine hydrolase. In order to do this, the DNAc of human CocE was incorporated into a type 5 adenoviral vector with a cytomegalovirus promoter (hdAD),[110] which could transfer the gene of the human CocE into rats for some days or weeks, generating notable and sustained quantities of the hydrolase in the liver,[111] and increasing the catalytic efficiency of the transferred protein, hdAD-CocH, compared to rat BChE, by up to 50,000 times.[106]

Other studies reported that administration of high doses of the vector raised the catalytic activity of CocH by up to 1 000 000 times with no apparent secondary reactions.[112-114] In fact, Murthy et al. reported that hdAD-mCocH vector transfer therapy did not cause adverse secondary effects on the functioning of the cholinergic system; subjects showed unchanged cognitive and motor functions.[115]

Administration of the hdAD-CocH vector (3mg/kg) to rats or mice reduced the half-life of cocaine and attenuated the cardiovascular effects caused by different doses.[111] Furthermore, it dramatically reduced the re-establishment of drug-seeking in the self-administration model (0.4 mg/kg) for up to six months,[111] however it did not alter water or food ingestion behaviors, or modify self-administration of amphetamines (0.05 mg/kg), nor did it reduce locomotive activity.[116]

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This suggests that the hdAD-mCocH vector did not alter motor efficiency or motivation related to drug-seeking; rather, its effect was specific to the reinforcement produced by cocaine.[64]

The transfer of the mutant of human CocH AME359 to rats through the hdAD-hCocH vector was recently reported. Administration of this vector in rats reduced the concentration of cocaine in plasma, prevented locomotive activity induced by cocaine, prevented the re-establishment of drug-seeking behavior for up to six months, and reduced fatality after an overdose (120 mg/kg).[114]

Other studies have reported the transfer of bacterial CocH through the use of bacteriophages. These are viruses that have the capacity to enter the bloodstream and easily cross the blood-brain barrier; they can tolerate a variety of adverse conditions such as extreme pH and treatment with nucleases and proteolytic enzymes.[117] Bacteriophages are therefore a good means by which to transfer exogenous molecules to the central nervous system such as CocH, which due to their size, the host's immune or enzymatic system may quickly return to circulation.

Howell et al. reported that transferring bacterial CocH through a bacteriophage to *Rhesus Macques* eliminated cocaine in the brain three times faster than systemic administration. This means of administration attenuated the reinforcing effects of cocaine[118] and avoided increases in blood pressure and cardiac frequency after administering an overdose.[105]

Rogers et al. achieved the expression of human CocE using protein III (pIII) and protein IX (pIX) within a bacteriophage. Both preparations, CocE-pIII and –pIX, were reproducible and generated high catalytic activity.[15]

Murthy et al. recently managed to transfer a mutated BChE to mice. The transfer through a viral vector raised the enzyme levels 1,000 times compared to normal levels, and increased the enzyme's catalytic capacity for months, capable of eliminating cocaine in a matter of seconds after its appearance in the bloodstream. Furthermore, the mutated BChE was capable of attenuating place preference and reducing blood pressure and fatality induced by overdose (80 mg/kg).[119]

Dual therapy

One of the main effects of administering cocaine overdoses (100-120 mg/kg, ip.) is permanent damage to the liver and muscles.[112] Individual therapies such as administration of human CocE (0.3 or 1 mg/Kg) or of monoclonal antibodies (10 or 20 mg/kg), or immunization with an immunogenic conjugate capable of producing antibodies against cocaine, have not yet been able to avoid these alterations. It has recently been reported that treatment with a combination of these therapeutic agents (enzyme, 1mg/kg-antibody, 8mg/kg, enzyme, 1 mg/kg-100 μg KLH-Norcocaine) provided complete protection to the liver and muscles.[112,113] It also completely blocked locomotive stimulation caused by 10mg/kg of cocaine,[21] which suggests that the combination of different therapies could increase protection against the psychostimulant actions of cocaine and extend its use into humans as support therapies for maintaining abstinence.[120]

DISCUSSION AND CONCLUSION

As mentioned previously, there has been a lack of an effective pharmacological therapy to date against the effects caused by cocaine,[1,2] especially in situations of intoxication by overdose. One therapeutic option is the use and validation of new alternative therapies.[3,4]

Taking overdoses proves fatal for a high percentage of cocaine addicts, as they cause cardiovascular and cerebral alterations, convulsions, and/or death. As such, based on the urgent need for an alternative therapeutic strategy, validation of the use of enzymes (BChE, CoCH, and bacterial CoCe) capable of significantly reducing dosage levels (even of lethal levels of cocaine) both in the bloodstream and the brain,[9,10] will provide emergency services with a unique therapeutic tool which will allow them to effectively reduce the lethal effects of overdose.[121] As well as its use in overdose situations, studies in animals allow the extension of these enzymes into potential therapeutic use in humans in order to quickly deactivate cocaine and develop treatments to avoid relapses and maintain abstinence.[122,123]

Phase I clinical studies have shown that the transfer of pure or recombinant (TV-138) human BChE into healthy subjects was a well-tolerated and safe therapy.[124] Treatment with different doses (50, 100, and 300mg) of BChE-TV-138 facilitated abstinence in patients dependent on cocaine, reduced its use, and attenuated subjective reinforcing effects caused by the drug.[125,126]

Although these studies would suggest that a therapy based on the use of human BChE is safe and could be useful in maintaining abstinence in dependent subjects, as is the case with other therapies such as active or passive vaccination, this therapy also has certain limitations: 1) its efficiency depends on the enzyme remaining in the bloodstream, 2) it is a therapy that can only temporarily avoid the drug crossing the blood-brain barrier, not for prolonged periods of time, 3) its use is therefore restricted to certain populations of subjects, particularly those who are in situations of intoxication by overdose.

In this sense, future studies need to assess the effectiveness and biological safety of using such a therapy, together with pharmacological, immuno-pharmacological, or psychological therapies.

This bibliographic review also has certain limitations: a) the bibliographical search did not widen to other search engines such as Biological abstracts, Google Scholar, Live

Search Academic, etc., b) truncation was not carried out on the descriptors used, c) no review was carried out of the bibliographical references of the articles included in the review, and d) the number of works aimed at describing the use of this therapeutic strategy in humans in cocaine overdose situations is small. All of these factors limit the conclusions drawn here.

These studies suggest that an increase in the catalytic activity of the enzymes BChE and hCE could be a useful strategy to develop an alternative therapy to treat patients in conditions of cocaine overdose toxicity.

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Conflict of interest

The authors do not declare any conflict of interest.

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