Sub-lethal toxicity and elimination of the cocaine metabolite, benzoylecgonine: a narrative review

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Abstract: Cocaine abuse is a serious global public health and social problem, and cocaine detoxification remains a challenge. Benzoylecgonine (BE) is the main toxic metabolite after cocaine consumption, with a longer retention time in the body and environment than cocaine itself. According to many studies, the toxicity of BE to humans is as significant as cocaine itself. Moreover, BE is recognized as an addictive drug contaminant in the environment, especially the freshwater system, leading to worries of its ecotoxicity. Extensive studies on the adverse effects of BE on both humans and ecology have been conducted, showing a marked sub-lethal toxicity of BE to diverse organisms. To eliminate BE *in vivo* and *in vitro*, various elimination methods have been developed and their BE removal capacity were evaluated. In this review, we aimed to summarize information in the literature to understand better BE toxicity and elimination that may facilitate the clinical treatment of cocaine abuse. By studying the critical role of BE in cocaine abuse, we propose that the ideal treatment for cocaine abuse should not only detoxify cocaine itself but also remove or degrade BE. Emphasizing the necessity of developing effective BE elimination methods is significant for the development of potential clinical treatments and environmental protections.

Keywords: Benzoylecgonine (BE); cocaine abuse; toxicity; ecotoxicity; elimination methods

Introduction

Cocaine abuse remains a significant worldwide public health problem with serious socio-economic consequences. According to the latest World Drug Report (1), 0.4% of the global population aged 15–64 (around 19 million people) reported having used cocaine in 2018. In addition to its intense excitatory effect on the nervous system, cocaine has non-negligible adverse effects on the cardiovascular, gastrointestinal, respiratory, musculoskeletal and renal systems (2,3). After consumption, approximately 40% of cocaine is rapidly biotransformed into inactive metabolite ecgonine methyl ester (EME) by plasma enzyme butyrylcholinesterase (BChE) and liver enzyme
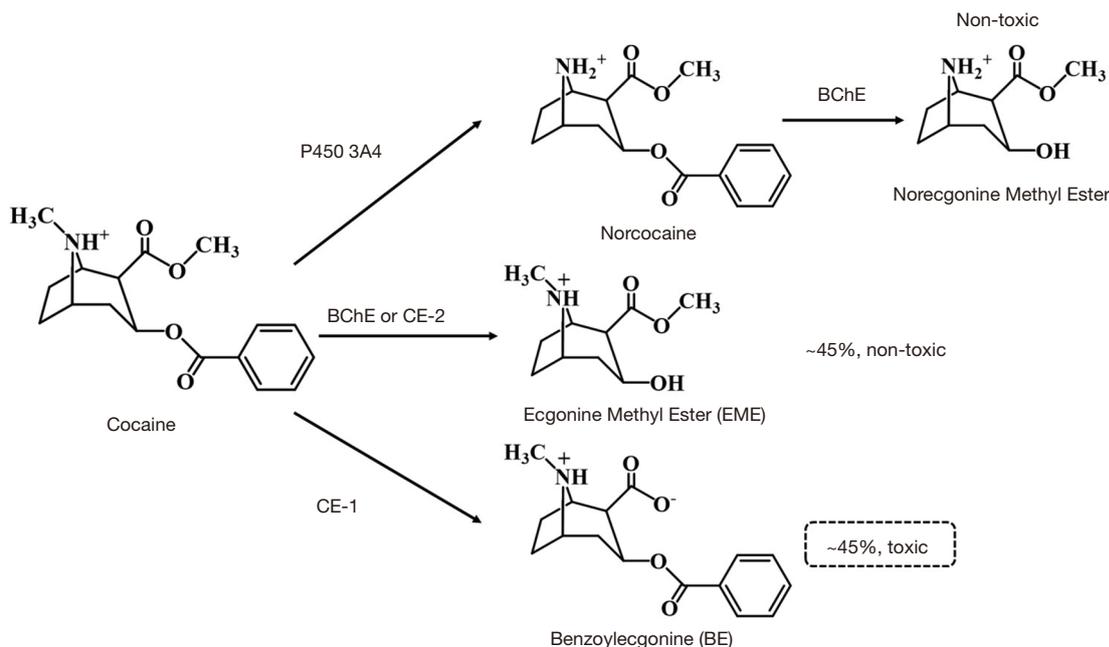
Figure 1 Benzoylecgonine is the main toxic metabolite of cocaine. Approximately 45% of cocaine is hydrolyzed to benzoylecgonine (BE) by carboxyesterase-1 (CE-1) in liver; 40% of cocaine is biotransformed to inactive metabolite ecgonine methyl ester (EME) by plasma enzyme butyrylcholinesterase (BChE) and liver enzyme carboxyesterase-2 (CE-2).

carboxyesterase-2 (CE-2), while 45% of cocaine is hydrolyzed to a toxic metabolite benzoylecgonine (BE) by carboxyesterase-1 (CE-1) in the liver (*Figure 1*) (4).

BE is an active metabolite with sub-lethal toxicities on multiple targets. A previous *in vivo* study showed that BE is a stronger vasoconstrictor than cocaine itself (5), hence the cerebral arteries are more sensitive (6). According to another study, the incidence of seizure is highly related to the presence of BE (7). Also, the latest research has found that even low concentrations of BE can significantly change the expression levels of proteins related to calcium homeostasis and oxidative emergency (8). These studies pointed out that BE has remarkable sub-lethal toxic effects *in vivo*.

Moreover, BE can remain in the body for a considerably longer period compared to the parent drug and can be detected in urine up to 4 days after last cocaine use. For this reason, BE is commonly used as a marker to screen individuals for a history of cocaine consumption or estimate the scale of the cocaine market (9). BE is poisonous and exhibits durable influences, which means that the removal or degradation of it into biologically inactive molecules is critical for cocaine detoxification.

BE is often excreted from the urine as one of the major substance forms of cocaine, and the environment is the ultimate destination. Unlike cocaine, BE is an amphoteric ion which is extremely stable in the human body and the natural environment (10). Consequently, the expelled BE is often detected in urban water systems in western and central European cities (1). Based on a wastewater analysis provided by Sewage Analysis CORe group Europe (SCORE), BE was found in the wastewater in 136 cities (150 sites) in Europe. In 2019, the amounts of population-normalized mass loads identified reached 225 mg/day per 1,000 inhabitants (1). According to more accurate reports, BE concentrations in the inlets and outlets of wastewater treatment plants (WWTPs) reached 7,500 and 3,425 ng/L, respectively (10,11). BE then enters the receiving water bodies including surface water, ground water and even drinking water sources, as proven by several monitoring studies. Even if BE concentration in the natural environment is low (for instance, 316 ng/L in one study) (10), the potential adverse effects on non-target organisms cannot be ignored. A mixture of illicit drugs containing 300 ng/L BE could alter the oxidative status of the zebra mussels, causing genetic damage and oxidative stress (12,13). A more recent study

on Mediterranean mussels found that 500 ng/L cocaine and 20 ng/L BE induced an oxidative stress situation both independently and in the mixture (14). The eco-toxicity of BE on diverse model aquatic species was evaluated at concentrations similar to those detected in surface waters (13,15-17). Increased evidence has confirmed that BE exhibits significant toxic effects on aquatic animals and plants. More importantly, it is reasonable to speculate that sustaining BE exposure in the environment could influence animals, plants, crops in the food chain and ultimately influence humans.

Given the sub-lethal toxic effects of BE on humans and the environment, there is a pressing need to identify efficient methods to either adsorb or degrade it *in situ*. Techniques such as active sludge reactors, reactive barrier, Fenton reaction, hydrogen peroxide (H_2O_2) treatment, ozone oxidation, and enzymatic degradation have been developed to transform BE into non-toxic products (18,19). Multiple technologies are usually incorporated to increase the removal efficiency against illicit drugs and their metabolites during water treatment (19). In recent years, enzymatic degradation is a useful strategy for BE detoxification both *in vivo* and *in vitro* (18). The effective enzymes against BE are suitably used in environmental protection by developing transgenic plants. BE elimination for humans and the environment has hopefully attracted attention and become a key aspect in cocaine abuse treatment.

The purpose of this study was to review the current literature concerning the toxicity of cocaine's principle metabolite, BE, as well as its elimination methods both inside and outside the body. This study aimed to describe possible solutions to prevent further toxic effects of BE during cocaine detoxification treatment. The discovery and development of highly effective methods for cocaine detoxification treatment are critical.

We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/apm-21-243).

Methods

The current review was carried out by an inclusive search in PubMed, using any combination of the following search terms: "benzoylecgonine", "cocaine abuse" and "toxicity". The search was not limited to either English-language articles or publication date. Ninety-two articles were found and evaluated for inclusion independently by

both authors and journals. Seventy-seven articles with strong relationships were selected for detailed evaluation. We did not assess the quality of the included studies. After the search, we constructed a list depending on the areas presented in the included studies. The first authors conducted an initial screening of the included studies by reading the titles and abstracts and decided whether they were relevant for our purposes. The entire team then carried out a second screening of the full-texts. Ultimately, detailed information was selected and presented here.
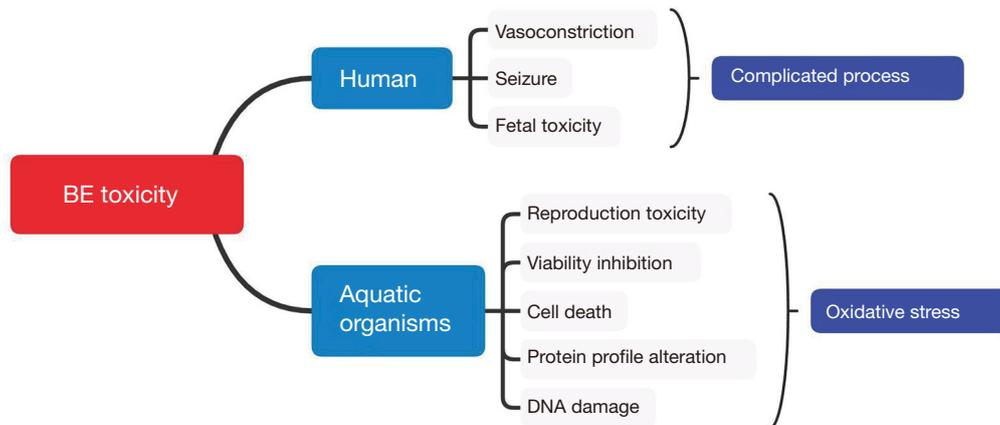
Toxicity of BE

Toxicity of BE in humans

Cocaine toxicity has been studied extensively, as has been shown in previous reviews (3), yet BE toxicity has been less explored. However, according to limited toxicology studies, BE exhibits different mechanisms of adverse effects on the cardiovascular system, nervous system and infants (*Figure 2*). Importantly, BE seems more toxic to certain organs such as cerebral arteries and infants. Moreover, chronic cocaine toxicity might be due to the presence of its toxic metabolite BE. Thus, it is necessary to re-estimate the toxic effects of BE *in vivo*.

Vasoconstriction

Clinically, cocaine is a vasoconstrictor. However, it has been reported that BE has more vasoconstriction effects compared to the parent drug and other drugs such as norcocaine and norepinephrine (5). BE has been shown to cause a significant decrease in the arterial diameter of isolated cerebral arteries of cats and fetal sheep (6,20,21). Cerebral artery segments are more sensitive to BE than cocaine and other cocaine metabolites (6). A previous *in vitro* study has demonstrated that BE cause a 30.1%±4.96% decrease in arterial diameter of at a concentration of 10^{-5} M (20).

Furthermore, a study has suggested that the constricting effect of BE is mediated via α1-adrenergic receptor stimulation (6), which is identical to the mechanism of cocaine-induced vasoconstriction. However, another study pointed out cocaine and BE exert vasoconstrictor effects through different mechanisms. This study reported that cocaine primarily activates adrenergic nerves and receptors, coupled with intracellular Ca^{2+} store activation, whereas BE seems to cause an influx of extracellular Ca^{2+} to increase the intracellular Ca^{2+} level (21). Cocaine was also reported to cause vasoconstriction by stimulating the sympathetic

Figure 2 Sublethal toxicity of BE to human and aquatic organisms. BE shows strong vasoconstriction effect in body, induces seizures and fetal toxicity in mechanisms that are different to cocaine. BE also has significant adverse effects on aquatic animals and plants, such reproduction toxicity, viability inhibition, cell death effect via inducing oxidation stress.

nervous system, followed by decreasing the reuptake of neurotransmitters including dopamine, epinephrine and norepinephrine (22). BE induced vasoconstriction is mediated by several complicated mechanisms that require further investigation. Furthermore, BE-induced vasoconstriction may be involved in other sub-lethal toxicities such as neurotoxicity and cardiovascular disease.

Chronic neurotoxicity

Cocaine-induced seizures commonly occur after cocaine consumption and are characterized by intense brain activity and repeated or rhythmic jerking muscle movements. Seizures have always been considered one of the serious effects of cocaine poisoning, in addition to psychosis and cardiovascular effects (2,23-27). Cocaine is a psychostimulant and psychotomimetic compound (28) that may induce acute and chronic psychiatric and neurological effects, including psychosis, agitation and seizures (29). The mechanisms underlying the neurotoxicity of cocaine are not fully understood since several intracellular biochemical processes appear to be involved (30-32). However, BE is assumed to be responsible for seizures, as cocaine only has a 6 hours half-life in the body, whereas seizure usually occurs later after cocaine intake (7,33). Scientists assume that BE-induced seizures occur via different mechanisms (26). Compared to equimolar amounts of cocaine, BE-induced seizures occur more frequently and have a significantly longer latency (7). In behavioral studies of rats, the frequency of seizures and the length of latency were directly

related to the dose of BE, with the frequency of seizures increasing from 33% to 86%, as well as a significantly decreased latency when the dose of BE was increased from 45 to 180 μg (33).

Fetal toxicity

BE could also inhibit embryos from developing into blastocysts at one or two cellular stages (34). Also, BE causes maternal and fetal hypertension (35). This phenomenon may be correlated to the afore mentioned vasoconstrictive effect of BE, and in the infant's brain, it could also lead to ischemic events or strokes, resulting in permanent neurological damage (33). A previous study has shown that infants and neonates exhibit signs of "neuroexcitation" following exposure to cocaine. However, BE was found in the urine and not cocaine. Cocaine was only detected in the urine of lethargic newborns (36). Therefore, some studies hypothesized that BE might cause behavioral changes in newborns and their hypotheses had been confirmed (37). Due to the incomplete development of the blood-brain barrier, the effect of BE on the fetal brain may be greater than the maternal effects. In pharmacokinetic studies, the concentration of BE in the fetal brain was higher than in the maternal brain (38-40), which is consistent with the differences in fetal and maternal bodies (41). A clinical study demonstrated that BE could persist in the urine of neonates for up to 48–120 hours after exposure but only persists for 27 hours in the urine of adults (41). The placenta may cause this phenomenon since BE is retained by the placenta and then subsequently leaches

into the fetal and maternal circulation (42).

Ecotoxicity of BE

Toxicity to freshwater animals

Parolini *et al.* assessed the consequences of BE exposure on *Daphnia magna*, a toxicological test species for water systems. The swimming activity of individuals and the reproduction of the population were investigated at two concentrations of BE (0.5 and 1.0 µg/L), which are similar to the levels previously detected in aquatic ecosystems (10). At high BE concentrations, the activity of *D. magna* showed a 5% reduction; however, the swimming velocity increased marginally (15). The chronic toxicity test showed that the total number of offspring (43% and 39% lower) and parthenogenetic cycles (45% and 21% lower) were significantly decreased at both concentrations of BE, respectively, indicating the strong reproductive toxicity of BE on *D. magna* (15). BE exposure also induced a marked increase in apoptotic or necrotic cell frequency at a concentration of 1.0 µg/L, showing values of up to 2.5- and 3-fold higher than the control (17,43).

BE exposure also induced a notable increase in cell mortality. In zebrafish embryo cells, cell mortality was significantly increased at BE concentration of above 0.4 nM (43). In *C. elegans* cells, according to the cell mortality results at different BE concentrations, the EC_{50} of BE was estimated as 0.6±0.1 ppm (44). A notable increase in the size of lipid droplets of *D. magna* at their juvenile stage was observed after BE exposure at concentration of 3.5×10^{-3} M. The accumulated lipid droplets indicated abnormal lipid synthesis or metabolism (44).

Furthermore, a proteomics study showed that exposure to BE for 14 days changed the protein profile of the gills of *D. polymorpha* (8). Diverse proteins involved in different systems had also shown alteration. Two actin isoforms and another protein, moesin, which is related to cytoskeleton structure, were also over-expressed at a BE concentration of 1 µg/L (8). The normally low-expressed actin isoforms take part in multiple important cell survival processes, such as structure conservation, motility, cellular organization and intracellular transport (45). Moesin is an integrin that plays biological roles in regulating basic cellular functions, including motility, adhesion, cellular signaling and cellular morphology (46). Exposure to BE also affected calcium homeostasis. The precursor of calreticulin was found to be over-expressed at the tested BE concentrations.

Calreticulin forms calnexin/calreticulin complexes and promotes proper folding, assembling and maturation of proteins (8). Proteins affecting apoptosis, like Galectin 7, were over-expressed at even lower concentrations of BE (8,47). Adenosyl-homocysteinase was over-expressed at two BE concentrations, affecting amino acid metabolism (8,48). The expression of two enzymes involved in glycolysis, namely enolase and GAPDH, were down-regulated after BE exposure, suggesting that BE may reduce the tolerance of zebra mussels to environmental stresses by decreasing energy metabolism (8,49). Low expression of EF-2, a protein involved in protein biosynthesis, was speculated to disrupt the metabolic pathways of zebra mussels and subsequently reduce the resistance to environmental changes (8,49).

Regarding another aquatic species, Parolini *et al.* found that exposure to a low concentration of BE (0.3 µg/L, respectively) would significantly alter the protein profile of zebrafish embryos during the early developmental period (96 hours after fertilization). Proteins belong to eye lens constituents, lipid transport and energy metabolism were also significantly altered in the BE group and the cocaine and EME groups (16). Also, exposure to high BE concentration (1 µg/L, respectively) had additional effects on proteins involved in lipid metabolism and oxidative stress response (16). This alteration was only observed for BE and EME, not cocaine (16).

Not only proteins but DNA was also be damaged due to BE exposure. A significant increase of DNA fragmentation in cells from *D. polymorpha* was observed by measuring the value of ligase detection reaction at BE concentrations of 0.5 and 1.0 µg/L, showing values 52% and 60% higher than the control, respectively (17). Significant DNA strand break was also observed in embryo cells at tested concentrations from 0.04 to 40 nM (43). Regarding fixed DNA damage, apoptotic, necrotic and micronuclei frequency increased, which followed both time and concentration dependencies (17,43).

Solid evidence has suggested that the adverse effects to macromolecules are mainly caused by BE-induced oxidative stress. In 2013, Parolini *et al.* investigated BE-induced oxidation on the freshwater mussel, *D. polymorpha*. In their study, the neutral red retention assay showed a significantly decreased trend of lysosomal membrane stability at both tested concentrations (0.5 and 1.0 µg/L, respectively) in a time and dose-dependent manner (17), suggesting a situation of general cellular stress likely linked to the induction of oxidative stress (50). For detailed biomarkers, the activities of three antioxidant phase I enzymes, namely

catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), as well as the phase II detoxifying enzyme glutathione S-transferase (GST), were altered imbalanced depending on the dose (17). The activities of these defense enzymes were also imbalanced in zebrafish embryo cells after BE exposure in a concentration range of 0.04 to 40 nM (43). Significant increases of SOD and GPx were recorded at 4 nM (~1.16 µg/L) (43). Clearer patterns of these oxidative status biomarkers were observed on *D. magna* at consistent BE concentrations. The reactive oxygen species (ROS) increased 13% while antioxidant GPx increased 68% (15). The detoxifying enzyme GST activity increased 80% and 46% at 0.5 and 1.0 µg/L of BE, respectively (15). The indirect evidence, obtained by measuring the lipid peroxidation (LPO) and protein carbonyl content (PCC), also indicated that substantial oxidative damages were caused after BE exposure (15,17,49,51,52). Taken together, BE has been shown to exhibit toxic effects on freshwater animals by elevating ROS levels and interrupting the balance of the antioxidant system. These results suggest that BE-induced oxidative stress may be the major reason for the toxicity of aquatic organisms.

Toxicity to freshwater plants

The toxicity of BE to freshwater plants has been less studied. However, based on a few studies, BE exposure caused developmental inhibition for certain plants. It was found that BE exposure caused a decrease in the viability of fern cells. After 48 hours of BE exposure (in a concentration range of 0.001 to 0.01 µg/L), the mitochondrial activity of *P. setiferum* fern spores were significantly reduced; however, when the BE concentration increased to a range of 0.1 to 10 µg/L, the mitochondrial activity gradually restored to a normal level. In contrast to the ambiguous result of mitochondrial activity, an approximately 20% reduction of DNA amount was observed at all test concentrations (53).

Furthermore, BE exposure had a 10% growth-inhibiting effect on algae *R. subcapitata* at concentrations of 3.5×10^{-3} M (44). A previous study had shown that at concentrations of 0.1 M or 3.29%, BE completely inhibited *Lupinus albus* growth (54). Exposure to BE also induced a high percentage of micronucleated cells in *V. faba* root tips (44).

Elimination of BE

Biodegradation via activated sludge reactor and reactive barrier

Conventional activated sludge (CAS) reactors have been widely used in WWTPs for decades (19,55). A laboratory study has demonstrated that BE (in the range of 500–4,000 ng/L) can be efficiently removed in 24 hours using the biological reactor (55). Biodegradation by heterotrophic bacteria contributed 91–95% of BE removal efficiency since the adsorption, and other abiotic processes were always low (55). Therefore, the activated sludge reactor is commonly used to eliminate BE and other illicit drugs or their metabolites in the water treatment process, combined with pre-treatment and other methods.
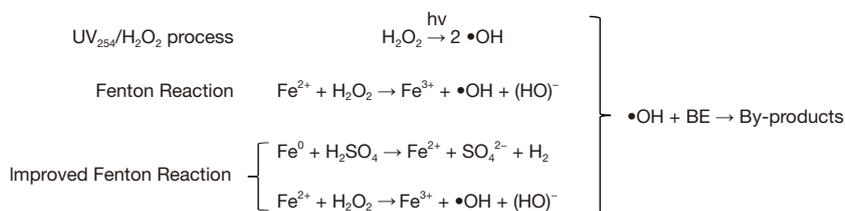
The reactive barrier is another low-cost BE clearance method. The reactive barrier is set at the bottom of an infiltration basin to promote the adsorption of BE, and consists of aquifer sand, vegetable compost, clay and iron oxide dust (56), and exhibits notable BE removal efficiency. However, the disadvantage of this method is that it takes a long time to completely remove BE, taking about 48 h in the test (56).

Chemical radical reaction

Adding H_2O_2 to the wastewater to generate •OH radical could also effectively remove BE (57-60). The formation of •OH by either continuous exposure to a UV_{254} lamp or a Fenton reaction is commonly used, with the •OH radical freely attacking the dissolved organic matter (*Figure 3*) (57). A study has reported that using the UV_{254}/H_2O_2 process, and staying in the microcapillary film array photoreactor for a few seconds or minutes could effectively remove BE. The Fenton reaction is one of the advanced oxidation processes, which can be used for pretreatment before biological level treatment and can also be used as a tertiary treatment process (58). Chemical reactions that occur in the Fenton reaction or improved Fenton reaction generate the •OH radical, effectively removing BE with an efficiency of approximately 90% (59). Another study explored the Photo-Fenton reaction by using Fe_2O_3/SBA-15 as a heterogeneous catalyst, and the removal efficiency of BE reached 92% (58). However, the researchers found that the products (peroxides, oxidant by-products) polluted with river water may cause greater toxicity (44,58,60). Therefore, BE by-products produced by peroxide may still exhibit potential toxicity, and this treatment process requires further treatment before discharge.

Oxidation by ozone disinfection

Ozone oxidation has been mostly applied in drinking

$$UV_{254}/H_2O_2 \text{ process} \qquad H_2O_2 \xrightarrow{h\nu} 2 \;\bullet OH$$

$$\text{Fenton Reaction} \qquad Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + (HO)^-$$

$$\text{Improved Fenton Reaction} \left\{ \begin{array}{l} Fe^0 + H_2SO_4 \rightarrow Fe^{2+} + SO_4^{2-} + H_2 \\ Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + (HO)^- \end{array} \right.$$

$$\bullet OH + BE \rightarrow \text{By-products}$$

Figure 3 Process/reaction generate •OH radical effectively remove BE, but produce by-products which may cause more toxicity.

Table 1 Comparison of BE removal efficiency in four WWTPs with different processes

WWTP	Primary	Secondary	Tertiary	Removal efficiency
1	Sedimentation	CAS with step feed process	No	95%±4%
			DAFF+Cl$_2$	99%±0.2%
2	No primary sedimentation	SBR	UV	42%±20%
3a	Sedimentation	CAS with MLE	Cl$_2$	82%±11%
3b	Sedimentation	4-stage Bardenpho +MBR	UV	87%±9%
4	Sedimentation	IFAS with MLE	Cl$_2$	87%±7%

WWTP, wastewater treatment plant; CAS, conventional activated sludge; DAFF, dissolved air flotation–filtration; SBR, sequencing batch reactor; UV, ultra violet; MLE, modified Ludzack-Ettinger; MBR, membrane bioreactors.

water treatment plants (DWTPs), and the efficiency of BE removal has been reported. The first stage of treatment includes chlorination, coagulation/flocculation, and sand filtration provides 9% BE removal efficiency. The second stage uses ozone disinfection and oxidation increases efficiency to 43%. The third stage use granulated activated carbon filters and the removal efficiency reached 72% (61). The overall BE removal efficiency using this three-step clearance was 89%. Researcher believes that the low removal efficiency might be due to the absence of ozone-reactive sites and electron-absorbing groups in ozone (61), and similar results were obtained in a study later conducted by another group (62). However, in this study, BE could still be detected in the treated drinking water with an average concentration of 45 ng/L (the maximum concentration was 130 ng/L). The study continued to carry out toxicological experiments to prove that it was not toxic to humans at this concentration (61). However, according to recent investigations, BE was still not removed effectively in the DWTPs (63).

Integrated process

Most WWTPs will combine multiple technologies to remove drugs. Recently, a long-term monitoring program was undertaken in Australia to characterize the fate of emerging drugs of addiction including BE, the major metabolite of cocaine. Different secondary and tertiary treatment technologies were investigated to remove of BE in sewage (19). WWTP1 comprises a CAS step feed process consisting of anoxic and aerobic zones, which is then subjected to secondary sedimentation/clarification, with a BE removal efficiency of 95%±4%. Subsequent three-stage treatment of dissolved air flotation–filtration (DAFF) and chlorination increased the removal efficiency to 99%±0.2%, respectively. The treated water could be directly used for horticulture, public place irrigation, and non-potable water purposes (19). The remaining WWTPs adopted a sequencing batch reactor (SBR)/Modified Ludzack-Ettinger (MLE)/4-stage Bardenpho with membrane bioreactors (MBR), or integrated fixed-film activated sludge (IFAS) process followed by UV disinfection or chlorination treatment, which exhibited insufficient BE removal efficiency (19). As shown in *Table 1*, this work enables a performance comparison between multiple combined wastewater treatment processes.

Enzymatic degradation

In previous studies, a few efficient and stable cocaine-

metabolizing enzymes were designed and developed based on human BChE or bacterial cocaine esterase (CocE), which showed rapid detoxification efficiency against cocaine (64-70). For the clinical treatment of cocaine overdose and addiction, using an efficient metabolic enzyme is considered a promising strategy (71-74). However, CocE and its mutants showed no activity against the toxic metabolite BE while only human BChE showed activity. Chen *et al.* demonstrated for the first time that BChE could hydrolyze BE into the non-toxic biological metabolites, ecgonine and benzoic acid. To improve the efficiency of BChE, their study constructed mutants to increase the efficiency of hydrolyzing cocaine, and at the same time, increase the degree and rate of BE degradation *in vitro* and *in vivo* (18). In the study, two mutants [A199S/S287G/A328W/Y332G mutant (named E14-3) and A199S/F227A/S287G/A328W/Y332G mutant (named E12-7)] were shown to have significant increases compared with wild-type BChE (18). Furthermore, *in vivo* experiments verified that E12-7 accelerated BE hydrolysis to ecgonine in a dose-dependent manner (18). The BChE-FC fusion or catalytic antibody analogs fusion proteins were further constructed to extend the time of action in the organism. Researchers believe that other mutants with better catalytic activity would be constructed through continuous research (18). The enzymatic degradation of BE seems to be an effective and eco-friendly elimination method that offers potential in the clinical treatment of cocaine overdose and environmental governance.

Discussion

BE is the main toxic metabolite of cocaine after a dose consumption. It has a similar molecular structure to cocaine, which is the major reason for long-term toxicities on the cardiovascular system, central nervous system, fetus, etc. In fact, BE causes more notable decreases in arterial diameter, suggesting it is a more potent vasoconstrictor than cocaine and other drugs, and as a result, cerebral artery segments showed higher sensitivity to BE than cocaine itself. Also, BE-induced seizure has a longer latency and frequency, and thus, seizure incidence after cocaine consumption is supposed to be primarily caused by BE. BE has a strong inhibitory effect on embryos in the early stages of development. Also, BE causes hypertension for both the mother and fetus, which may be correlated with the strong vasoconstrictive effect. In some studies, BE appears to pass the blood-brain barrier of the newborn more easily, which causes more fetal toxicity.

Moreover, *in vitro* experiments showed that exposure to BE decreases cell viability and increases cell mortality, and 20 μM of BE exposure results in cell death. At the same time, BE can exist in the body for a relatively long time and is used as an indicator of cocaine consumption. Thus, the toxicity effects of long-term BE exposure in the body should be investigated more extensively.

BE is one of the major illicit drug metabolites found in the environment, primarily the aquatic system. Considering the increased use of cocaine worldwide and an incessant BE input in freshwater, a consequent increase in concentration is expected. Exposure of BE seems a sort of pseudo-persistence to aquatic organisms. Numerous studies have been carried out to evaluated the critical aspect of the environmental risk of BE. Notable adverse effects, such as abnormal synthesis or metabolism of lipids, genetic damage and protein expression alteration, are related to oxidative stress after BE exposure. Importantly, *in vivo* studies toward aquatic model species, the zebrafish embryos, showed that BE is more toxic than cocaine. These studies highlighted the relationships between biochemical, behavioral, and reproductive endpoints. In contrast, the studies concerning BE toxicity on plants are few, with growth inhibition being the major adverse effect. The use of BE-polluted surface waters as irrigation and drinking water sources should cause considerable anxiety of persistent exposure of BE to humans.

Fortunately, numerous methods of BE elimination have been developed and applied in different processes, including wastewater, drinking water, and clinical emergency treatment. WWTPs play a critical role in the removal of BE to minimize discharge into the receiving environment. Currently, the methods currently used in water treatment processes are activated sludge reactors, photochemical methods, reaction barriers, etc. The removal mechanisms involved are biodegradation, adsorption, photochemistry decomposition, Fenton reaction, etc. Several studies have shown that activated sludge processes with effluent disinfection, such as IFAS with chlorination and MBR with UV, were the most efficient in eliminating the targeted compounds than other more conventional secondary treatment processes. Additional tertiary treatments appear to be effectively achieve near-complete removal of BE and other drugs in recycled waters destined for agricultural irrigation and other regulated municipal uses. According to the extensive studies, it is highly recommended to apply a CAS step feed process consisting of anoxic and

aerobic zones, which is critical to reach an acceptable BE removal efficiency (>90%, respectively). Additional tertiary stage using dissolved air flotation–filtration (DAFF) and chlorination would increase the removal efficiency to nearly complete in WWTPs. However, DWTPs appear to be not entirely effective in eliminating BE, resulting in human intake. Current technologies in each step have limitations, and a fourth step with advanced technology should be applied for better clearance. Removal methods still require further improvement, and advanced technologies should be expanded globally. For clinical treatment of cocaine overdose, accelerating BE metabolism seems a promising therapy method. A recent study focusing on identifying and engineering the natural enzyme with inherent BE metabolism activity showed the prospective potential of enzymatic metabolism (18). Human BChE with inherent BE metabolism activity was redesigned to improve both activity and duration *in vivo*, the data showed a promising starting point of effective BE metabolism. Emphasizing that BE is a long-lasting toxic metabolite *in vivo*, ideal enzyme therapy for cocaine overdose should not only hydrolyze cocaine itself in a short period, but also can degrade BE into non-toxic molecules to prevent persistent toxicity. However, it is still extremely challenging to efficiently treat cocaine overdose using current enzyme products due to the enzyme activity and stability. We expect that new enzymes with higher BE removal efficiency *in vivo* could be developed for clinical use and environmental governance.

Conclusions

Findings from the present review showed that BE is a significant toxic metabolite to humans and the environment. To obtain a better understanding of the adverse effects of BE, more extensive studies focusing on the mechanisms of BE toxicity are needed in the future. However, according to current research, few studies investigated the potential effects of BE exposure on humans from the environment. Finally, the BE elimination methods with high removal efficiency are not well-established during water treatment, which may cause subsequent ecotoxicity. More importantly, the clinical treatment of cocaine overdose requires detoxification of cocaine and simultaneous degradation of BE.

Acknowledgments

Footnote

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