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Application of Tetrameric Recombinant Human Butyrylcholinesterase as a Biopharmaceutical for Amelioration of Symptoms of Acute Organophosphate Poisoning

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We present a procedure for optimizing the expression of recombinant tetrameric butyrylcholinesterase that enables large-scale production with the yield >30 mg/liter (>90 mg/roller bottle). Intravenous injection of the preparation significantly increased survival and decreased the severity of symptoms of poisoning with paraoxon, an organophosphorus toxin.

Key Words: *tetrameric recombinant human butyrylcholinesterase; paraoxon; prevention of poisoning; organophosphate toxins; biological drug*

Acute organophosphorus (OP) poisoning is one of the most frequent causes of mortality related to chemical poisoning (260,000 deaths annually) [5]. Most victims are residents of the Western Pacific Region, where OP poisoning accounts more 50% of suicide attempts. Accident OP poisonings associated with improper handling of pesticides during agricultural activities are also frequent. Numerous cases of OP application during military operations and terrorist acts also pose a serious threat and necessitate the development of highly effective methods for treating OP poisoning.

Modern therapy of OP poisoning is based on combined use of muscarinic receptor antagonist (atropine) and acetylcholinesterase reactivator (pralidoxime or obidoxime) [2]. Although this treatment is effective in some types of OP poisoning, it shows no positive effect in case of pesticide poisoning and does not prevent irreversible brain damage [3]. In this context, biological antidotes (bioscavengers) are of great interest, because they can irreversibly inactivate OP before they attack their biological target, acetylcholinesterase [7], and protect the nerve cells from damage by OP.

Human butyrylcholinesterase (hBCHE) is a natural bioscavenger in OP poisoning [14] and can inactivate a wide spectrum of OP [11]. The use of hBCHE improves survival rate during poisoning with warfare agents of type V [4,8] (>8LD_{50} in the case of VX gases) and type G [4,10] (> 5.5LD_{50} in case of soman), as well as with pesticides [4,9]. Moreover, the use of hBCHE allows preventing long-term side adverse of OP poisoning, including irreversible brain damage [12].

Despite unique therapeutic indices of hBCHE-based biopharmaceutical, its large-scale production is limited, as it needs to be isolated from human blood plasma, which makes it extremely expensive and unsafe because of the risk of viral contamination. The possibility of large-scale production of recombinant hBCHE was previously demonstrated for plants [4] and transgenic animals [6], but hBCHE in these systems is produced as a mixture of monomers and dimers characterized by extremely rapid excretion ($\tau_{1/2}$≈2 min) from circulation, unlike their natural tetrameric form.

Using an alternative approach to produce hBCHE in CHO cells and genetic constructs carrying hBCHE gene and a sequence encoding native tetramerization peptide, we achieved a high level of production of ex-

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