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REVIEW ARTICLE

MILITARY INCAPACITATING AGENT BZ (3-QUINUCLIDINYL BENZILATE) – PAST, PRESENT AND FUTURE

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Summary

The military incapacitating agent BZ (3-quinuclidinyl benzilate) is an anticholinergic compound that acts at both, the peripheral and central nervous system. Effects of the agent were discovered during the Cold War and BZ became one of the most potent anticholinergic psychomimetics, characterized by low effective doses causing long-term incapacitation. History, characteristics and potential use of BZ in behavioral research are discussed throughout this review.

Key words: acetylcholine; neurotransmission; behavior; learning; memory

INTRODUCTION

Military incapacitating agents are compounds which cause temporal physiological or mental impairment with a non-lethal effect under the effective dose. Accordingly, several agents, such as e.g. derivatives of lysergic or glycolic acid, meet the requirement to be highly potent and logistically feasible compounds, showing the effects mainly by disrupting the higher regulatory activity of the central nervous system lasting for hours/days with no serious life-threatening effect. Characterized by a therapeutic index of approximately 1000 and a strong central and peripheral effect, BZ seemed to be an ideal chemical incapacitating agent.

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HISTORY

The psychotomimetic effect of anticholinergic drugs is known from Antiquity. The first mentioning of Deadly Nightshade (*Atropa bella-donna*) and its effect comes from a Greek philosopher and naturalist, "father of botany", Theophrastus (371 – 287 BC). During Antiquity and the Middle Ages, atropine and other natural alkaloids were commonly used for religious ceremonies, sabbaths, or as a means of poisoners. Beginnings of medical use are dated to the 17th century. In the 1950s, a number of anticholinergic drugs including esters of glycolic acid were investigated for potential military and industrial use. Origin of Agent BZ can be attributed to increasing interest of US military in exploring chemical warfare agents which were non-lethal, but just "incapacitating". This demand was partly forced by public opinion asking for "War without death", as proclaimed in newspapers at the time. BZ was originally investigated for medication of gastrointestinal diseases as anti-spasmodic drug. Due to many side effects, tests were suspended after some time and the compound

was transmitted to the US military for potential use as an incapacitating agent. There is evidence that BZ was tested on human subjects in Utah under codename "Project Dork" in early 1960s and later in Hawaii during 1966 and 1967 [1]. BZ was subsequently weaponized until stocks were destroyed in early 1990s. Critics pointed to the unpredictable effects of BZ which could evoke aggression in passive personnel. Additionally, BZ was outstripped by more effective incapacitants as derivatives of fentanyl. Nowadays, BZ is commonly used for identification of M receptors via several laboratory techniques, mainly radio-ligand binding assay [e.g. 2, 3], and, due to its psychotomimetic properties, it has also been used to generate a cognitive deficit in animal models of neurodegenerative disorders, such as Alzheimer's disease [4-6].

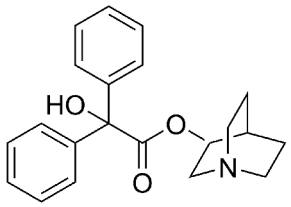

Figure 1. Chemical structure of Agent BZ (3-quinuclidinyl benzilate).

Characteristics of BZ

3-quinuclidinyl benzilate (QNB; Agent BZ; Agent Buzz; EA2277; Fig. 1) represents a non-selective, competitive antagonist of cholinergic receptors. Similarly as some nature alkaloids e.g. atropine or scopolamine, BZ competes with neuromediator acetylcholine at muscarinic receptors in both, central and peripheral sites. Thus, the action of BZ is reverse to an effect observed in nerve agent poisoning. While action of nerve agents is manifested by cholinergic overstimulation, BZ leads to a lack of cholinergic transmission, presented by e.g. Mydriasis, xerostomia, and tachycardia or flushing of the skin. The interaction of BZ and central cholinergic system is connected with psychotomimetic incapacitating signs such as e.g. confusion, disorientation or delusions. True hallucinations could be intensive resulting into irrational and unpredictable behaviour [7].

BZ is white, odorless crystalline solid which is absorbed mainly via oral route, or respiratory system when dispersed as aerosol. Incapacitating dose (ICt50) is approximately 110 $mg.min^{-1}.m^3$. Percutaneous penetration is considered when dissolved in appropriate solvent. BZ easily crosses the blood-brain barrier and interacts with all sub-types of muscarinic (M) receptors responsible for various central effects. Onset of anti-cholinergic signs is rapid, observed immediately after subcutaneous injection of high doses or several min after low doses [8]. Action of BZ is expected to be long-lasting with significant behavioral effects within 24 hours in rats [6]. A noticeable, but non-significant behavioral effect was observed even 7 days after administration [6]. The mean duration of incapacitation is considered to be about 70 hours in a man [9]. Action of BZ is biphasic [8]. The first stage, which lasts up to 4 hours in a man, is characterized by hyperactivity, observed also in experimental animals, muscle spasms, feelings of unrest and discomfort. The second stage, lasting up to 4 to 12 hours is connected with sedation and inactivity. Complex hallucinations continue 24 to 48 hours post exposure. Low effective dose (ED) is expected to induce incapacitation in a man – 0.01-0.006 $mg.kg^{-1}$ (ED, *i.m.*)[9].

Reversible inhibition of cholinesterase is the principle of effective symptomatic treatment. Physostigmine, a parasympathomimetic natural alkaloid derived from the African plant *Physostigma venenosum* (calabar bean), was the first antidote against BZ [9]. Due to a narrow therapeutic index, many side effects and a short-term duration, physostigmine was replaced with a safer acridine derivative, 7-Meota [10].

BZ in behavioral studies - impact on learning and memory

It is generally accepted that pharmacological intervention into central cholinergic system via anticholinergic drugs influences learning and memory [11-13]. The shortage of cholinergic transmission is connected with behavioral alterations and impairments of cognitive functions, resembling a typical accompanying sign of neurodegenerative diseases. Thus, anticholinergic drugs such as e.g. scopolamine, atropine, or trihexyphenidyl, are under the scope of scientists in the field of neuroscience, presenting potential pharmacological models of neurodegenerative disorders in laboratory animals [12, 14-18].

Several times, agent BZ has been used to generate an animal model for testing novel acetylcholinesterase inhibitors *in vivo* at the behavioural laboratory of the Department of Toxicology, Faculty of Military Health Sciences. BZ is usually administered to animals intraperitoneally, 30 min before the behavioral test. When treated, the *i.p.* injection containing therapeutic dose of the tested inhibitor follows 15 min after BZ. The effective experimental dose in Wistar rats performing Morris Water Maze was found to be 2.0 mg.kg^{-1} (Fig. 2). The Morris Water Maze is a common and widely used behavioral test for spatial learning and memory, requiring search for the hidden platform submerged in the circular pool [12, 19]. Agent BZ at the dose of 2.0 mg.kg^{-1} induces disorientation in performing rats, characterized by a prolonged path and escape latency with insignificant side effects as locomotoric and attentional disruption [6,20]. The behavioral alteration was found only when the substance was administered before training; well trained rats showed no alteration in the test of spatial memory. Concordant outcome was found in the passive avoidance, the behavioral task presenting fear conditioning that requires suppression of innate response. Rats administered with BZ before training entered the "forbidden" dark compartment in the 24 hour retention test, whereas rats administered 30 min before the retention test prevented entering the forbidden area, identically as control rats administered with saline. These results indicate that BZ impairs specifically the process of acquisition ("learning"), whereas retrieval of consolidated information ("long-term memory") is not affected. Indeed, the neurotransmitter ACh shows a biphasic effect, shifting between acquisition and retrieval in hippocampus [21]. Whereas high levels of ACh facilitate acquisition of new information ("learning"), impair consolidation and recall of previously stored memories [22]. Accordingly, cholinergic blockade via BZ (and possibly another anticholinergics) followed by decreased cholinergic transmission lead to deteriorated learning but not long-term memory, as observed. Thus, BZ seems to be an effective alternative to the most widely used scopolamine in behavioral tests focused on learning process in experimental animals.

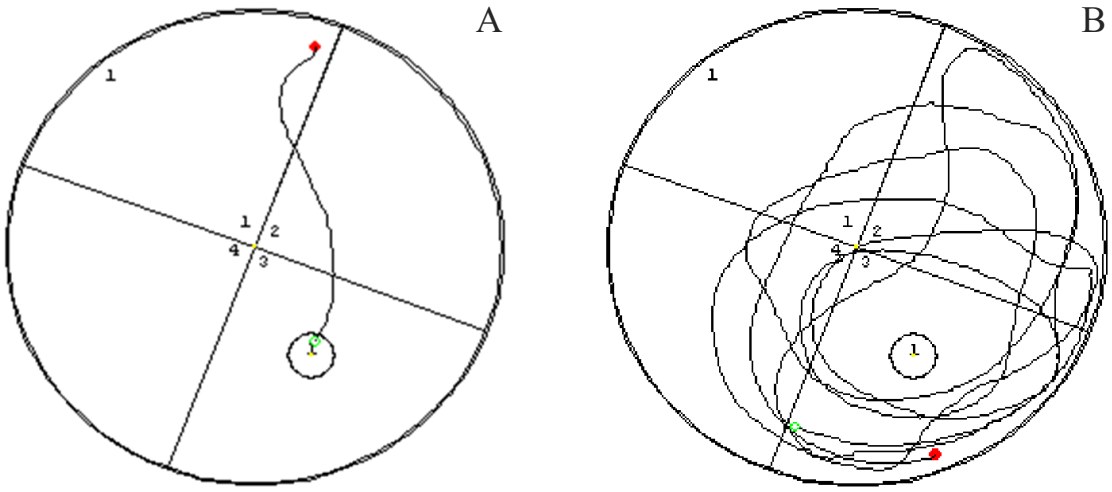

Figure 2. Total path of a rat pre-exposed to normal saline (A) and 2 mg.kg^{-1} of BZ (B) in the Morris Water Maze task, performed 30 min post intraperitoneal injection. Goal platform is identified as a circle in the middle of quadrant no. 3. *Illustrative image*.

CONCLUSION

Agent BZ was historically produced and stored as a potential military incapacitant in the US, though there is no direct evidence that it has ever been used. Nowadays, US stocks are officially destroyed. Nevertheless, the use of BZ could not be excluded e.g. by terrorist groups due to relatively easy availability. While the use of BZ as a military incapacitating agent seems to be history, the future lies in involvement of BZ into the research of cholinergic receptors and transmission as well as behavioral research.

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