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β-LACTAMASE INHIBITORS DISPLAY ANTI-SEIZURE PROPERTIES IN AN INVERTEBRATE ASSAY

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Abstract—Antibiotics containing a β-lactam ring (e.g. ceftriaxone) display anti-glutamate effects that underlie their efficacy in animal models of central nervous system (CNS) diseases [Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB (2005) Nature 433:73–77]. We hypothesized that the structurally related β-lactamase inhibitors (clavulanic acid, tazobactam)—which also contain a β-lactam ring—will mimic ceftriaxone efficacy in an invertebrate (planarian) assay designed to screen for anti-seizure activity [Rawls SM, Thomas T, Adeola M, Patil T, Raymondi N, Poles A, Loo M, Raffa RB (2009) Pharmacol Biochem Behav 93:363–367]. Glutamate or cocaine administration produced planarian seizure-like activity (pSLA). Glutamate- or cocaine-induced *p*SLA was inhibited by ceftriaxone, clavulanic acid, or tazobactam, but not by the non-β-lactam antibiotic vancomycin. The present findings indicate β-lactamase inhibitors display efficacy, and mimic ceftriaxone activity, in an invertebrate anti-seizure screen. These results suggest β-lactamase inhibitors—particularly ones such as clavulanic acid that display enhanced brain penetrability, oral bioavailability, and negligible anti-bacterial activity—might offer an attractive alternative to direct antibiotic therapy for managing CNS diseases caused by increased glutamate transmission and provide a solution to the growing concern that ceftriaxone will be of only limited utility as a CNS-active therapeutic because of its intolerable side effects.

Key words: clavulanic acid, tazobactam, ceftriaxone, β-lactam antibiotic, glutamate, planaria.

β-lactam antibiotics enhance cellular glutamate uptake through activation of glutamate transporter subtype 1 (GLT-1), a predominantly astrocytic transporter that is responsible for about 90% of glutamate uptake in the brain and widely recognized by neuroscientists as a promising target to manage central nervous system (CNS) diseases caused by excessive glutamate transmission. The representative β-lactam antibiotic ceftriaxone has already been successfully used as a pharmacological probe. It is active in preclinical models of CNS diseases such as amyotrophic lateral sclerosis, multiple sclerosis, stroke, depression, tolerance, addiction, and seizure (Rothstein et al., 2005; Chu et al., 2007; Lipski et al., 2007; Miller et al., 2008; Rawls et al., 2010a,b; Knackstedt et al., 2010; Sari et al., 2009). The main problem with ceftriaxone is that its broad-spectrum preclinical efficacy may not actually translate into a clinically useful CNS-active therapeutic. Its poor brain penetrability requires the administration of high concentrations to achieve anti-glutamate effects in animals and humans, a requirement that exacerbates the risk of adverse effects. For example, the standard ceftriaxone concentration of 200 mg/kg, given for 5–10 days, to achieve anti-glutamate effects in rats and mice equates to about 13 g/day in a human, approximately 5–6 times greater than ceftriaxone concentration prescribed for meningitis. It is not surprising that many patients receiving ceftriaxone to treat amyotrophic lateral sclerosis have discontinued its use due to intolerable side effects, an outcome that underscores the need to identify more patient-friendly therapies. The structurally-related β-lactamase inhibitor clavulanic acid may be a particularly attractive option. Clavulanic acid possesses a β-lactam ring, an apparent structural requirement for the anti-glutamate effects of the β-lactam antibiotics. It is also orally active and stable, with a bioavailability of 64–75%, a distinct advantage over ceftriaxone which is normally administered intravascularly or intramuscularly (Bolton et al., 1986). Clavulanic acid also displays excellent blood-brain barrier penetrability (CSF/plasma ratio of 0.25) and exhibits only negligible anti-bacterial activity (Nakagawa et al., 1994). A first step in determining if clavulanic acid should be explored as an alternative to direct ceftriaxone therapy for managing CNS disorders is to compare its efficacy with that of ceftriaxone in an animal model. Prior work indicates that ceftriaxone displays anti-seizure properties in mice (Jelenkovic et al., 2008). The goal of the present study was to investigate and compare the activities of β-lactamase inhibitors (clavulanic acid and tazobactam), β-lactam antibiotics (ceftriaxone), and non-β-lactam antibiotics (vancomycin) in an assay designed to screen for anti-seizure activity (Rawls et al., 2009). In addition, the effect of a GLT-1 transporter inhibitor, dihydrokainate, on glutamate- and cocaine-induced seizure like activity was investigated.

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Abbreviations: CNS, central nervous system; GLT, glutamate transporter; *p*SLA, planarian seizure-like activity.

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Table 1. Experimental design

Experiment	Drug(s)
1A	Ceftriaxone (0.01–25 mM) or water + Glutamate (3 mM)
1B	Ceftriaxone (0.01–25 mM) or water + Cocaine (3 mM)
2A	Clavulanic acid (0.1–5 mM) or water + Glutamate (3 mM)
2B	Clavulanic acid (0.0001–1 mM) or water + Cocaine (3 mM)
3A	Tazobactam (0.005–0.1 mM) or water + Glutamate (3 mM)
3B	Tazobactam (0.005–0.1 mM) or water + Cocaine (3 mM)
4A	DHK (0.01–1 mM) or water + Glutamate (3 mM)
4B	DHK (0.01–1 mM) or water + Cocaine (3 mM)
5A	Vancomycin (1–10 mM) or water + Glutamate (3 mM)
5B	Vancomycin (1–10 mM) or water + Cocaine (3 mM)

Each planarian was placed into a clear plastic petri dish containing a drug or drug combination and tested individually for *p*SLA for 5 min.

EXPERIMENTAL PROCEDURES

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply (Burlington, NC, USA) and tested within 3 days of receipt. Solutions of L-glutamic acid, cocaine, ceftriaxone, clavulanic acid, and tazobactam were prepared daily in distilled water. Planarian seizure-like activity (pSLA) was quantified as the number of C-like hyperkinesias during a 5 min drug exposure (Rawls et al., 2009). C-like hyperkinesias have been observed previously upon exposure to cholinergic, opioid, and dopaminergic agents, as well as upon cocaine exposure (Buttarelli et al., 2000; Palladini et al., 1996; Passarelli et al., 1999; Pagán et al., 2008). Drug combinations tested in each experiment are described in Table 1. Comparisons of group means (±SEM) were evaluated by one-way ANOVA followed by a Dunnett's post hoc analysis. $P<0.05$ was considered statistically significant. All experiments conformed to local and international guidelines on the ethical use of animals. Cocaine was obtained from the National Institute on Drug Abuse (Bethesda, MD, USA). Glutamate, vancomycin hydrochloride, clavulanic acid, tazobactam, and ceftriaxone sodium salt were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dihyrokainate was purchased from Torcis Biosciences (St. Louis, MO, USA).

RESULTS

Planarians exposed to water did not display *p*SLA. However, they did display concentration-related *p*SLA following an acute 5-min exposure to glutamate or cocaine (Table 2). Concentrations of 3 mM glutamate and 3 mM cocaine were selected for drug combination experiments. For combined administration, ceftriaxone (3, 5, 10, 25 mM) significantly inhibited glutamate- or cocaine-induced *p*SLA (Fig. 1A, B). Clavulanic acid (1, 3, 5 mM) attenuated glutamate-induced *p*SLA (Fig. 1C) (Fig. 2A), and a similar inhibition of cocaine-induced *p*SLA was detected, but at even lower clavulanic acid concentrations (0.005, 0.01, 0.05, 0.1, 1 mM) (Fig. 2B). Tazobactam (0.01, 0.05, 1 mM) also produced significant inhibition of *p*SLA induced by glutamate or cocaine exposure (Fig. 3A, B). Half-maximal inhibitory concentrations (IC_{50}) of the β-lactam compounds were (glutamate, cocaine): ceftriaxone (5.8, 24.5 mM); clavulanic acid (1.44, 0.010 mM); and tazobactam (0.018, 0.019 mM). Ceftriaxone administered by itself at concentrations of 0.01–25 mM did not produce *p*SLA, but it did cause *p*SLA at a concentration of 50 mM (8.5±1.56 pSLA/5 min). Clavulanic acid or tazobactam, when given alone, did not produce *p*SLA at the concentrations used here. Dihydrokainate (0.3, 1 μM) significantly inhibited glutamate-induced *p*SLA (Fig. 4A), and a similar inhibition of cocaine-induced *p*SLA was observed for dihydrokainate (0.03, 0.1, 0.3, 1 μM) (Fig. 4B). Dihydrokainate (0.01–1 μM) did not produce *p*SLA at the concentrations used in combination experiments, but it did induce *p*SLA at higher concentrations (10 μM, 3.2±0.56 *p*SLA/5 min; 30 μM, 6.7±1.22 *p*SLA/5 min; and 100 μM, 12.5±2.68 *p*SLA/5 min). The non-β-lactam antibiotic vancomycin (1, 3, 10 mM) did not significantly affect *p*SLA produced by glutamate [$F(3, 32)=1.958$, $P>0.05$] or cocaine [$F(3, 24)=0.697$, $P>0.05$] (data not shown).

DISCUSSION

The demonstration that clavulanic acid and tazobactam, as well as ceftriaxone, display activity against glutamate- and cocaine-induced seizure-like activity in invertebrates confirms the documented anti-seizure properties of ceftriaxone and indicates β-lactamase inhibitors may be capable of mimicking the efficacy of β-lactam antibiotics in animal models of CNS diseases caused by excessive glutamate activity (Jelenkovic et al., 2008). Prior work suggests the behavioral assay used here is predictive for anti-seizure activity (Rawls et al., 2009). For example, pro-convulsants (e.g. AMPA, NMDA, glutamate, cocaine) produce quantifiable seizure-like activity (pSLA) that is inhibited by clinically approved anti-epileptic agents (e.g. topiramate, valproate), but not by control drugs (e.g. propranolol) (Rawls et al., 2009). It is already documented that ceftriaxone inhibits seizures induced by the GABA receptor antagonist penteleyneterazole in mice (Jelenkovic et al., 2008). The present data indicate ceftriaxone efficacy extends to an invertebrate model of seizure. More importantly, anti-seizure activity was dependent on the presence of a β-lactam ring but not anti-bacterial activity. Three β-lactam-containing compounds—ceftriaxone, clavulanic acid, and tazobactam—were active in the assay whereas vancomycin, an antibiotic lacking the β-lactam ring, was inactive. There is one structural difference in the β-lactam core

Table 2. Planarians exposed to either glutamate or cocaine for 5 min display concentration-related *p*SLA

Drug concentration (mM)	pSLA(glutamate)	pSLA(cocaine)
0.01	3.4±0.5	0.7±0.9
0.1	11.4±1.5	2.5±1.2
1	18.5±0.6**	7.3±0.7
3	26.3±2.4**	23.4±1.2**
10	34.5±3.9**	29.8±0.8**

Control planarians exposed to drug-free water did not display *p*SLA. $n=6-8$ planarians per group.

** $P<0.01$ compared to 0.01 mM group.