Pro-convulsant effect of cefazolin sodium against pentylenetetrazol- or picrotoxin-induced convulsions in mice

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Cefazolin injection (3000 mg/kg, iv) in mice showed several behavioral excitations such as wild running, jumping, rolling, and finally undergoing severe convulsions followed by death. It’s lower doses (500-2000 mg/kg, iv) were unable to produce any convulsions or behavioral excitations in mice. However, cefazolin (500 or 1000 mg/kg, i.v.) when administered before different doses of pentylenetetrazol (PTZ; 40 or 60 mg/kg, ip) or picrotoxin (PTX; 4 or 8 mg/kg, ip.), it produced severe tonic-clonic convulsions in mice. The convulsions or behavioral excitations produced by 3000 mg/kg, iv cefazolin was also reversed by different doses of diazepam (0.5-2 mg/kg, ip) further proving the GABAergic modulatory effect of cefazolin. The results conclude the pro-convulsant action of cefazolin on PTZ- or PTX-induced convulsions, and further confirm the clinical reports.

Keywords: Cefazolin, Convulsions, Diazepam, GABA, Pentylenetetrazol, Picrotoxin

Cefazolin (3-[[5-methyl-1,3,4-thiadiazol-2-yl]thio]-methyl]-8-oxo-7-[2-(1H-tetrazol-1-yl) acetamido]-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid), a first generation parenteral cephalosporin, is the drug of choice for surgical prophylaxis1,2 and to treat various staphylococcal or streptococcal infections3,4. Various clinical evidences have indicated the ability of therapeutically important cephalosporin antibiotics like ceftriaxone, ceftazidime, cefepime to induce non-convulsive status epilepticus in patients with renal failure5,6, similarly use of cefazolin at higher doses results in several side-effects, including its ability to produce convulsions.

Many investigations are being carried out to elucidate the convulsive potential and the mechanism of action of these antibiotics in humans and experimental animals7-13. Previous work on the convulsant action of penicillins and cephalosporins attributed to the suppression of γ-amino butyric acid (GABA) system by these antibiotics14-16. Conforming with this hypothesis, in vitro studies with cultured neuronal and [3H] GABA binding indicated that β-lactams evoke seizure activity by suppression of the inhibitory postsynaptic responses17-19 mediated by GABA in various brain areas11,20. However, there is not much experimental evidence available regarding the convulsant ability of cefazolin in animal models. Therefore, the present study has been designed to explore the possible convulsant ability of various doses of cefazolin after its acute intravenous administration in mice, and to investigate its pro-convulsant effect on different doses (doses that are either subeffective or produced mild convulsions) of GABA antagonist viz. pentylenetetrazol or picrotoxin and also to examine the involvement of GABAergic mechanism in the cefazolin-induced seizures by using diazepam, a GABA antagonist.

Materials and Methods

Animals — Male Albino mice (Laka strain) weighing between 22–30 g bred in Central Animal House facility of Panjab University, Chandigarh were used. The animals were housed under standard laboratory conditions maintained under a natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 and 1500 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy Guidelines (icmr.nic.in/bioethics/INSA_Guidelines.pdf) for the use and care of experimental animals.
Drugs and treatment — The following drugs were used: Cefazolin sodium (Ranbaxy Research Laboratories, Gurgaon, India), diazepam (Ranbaxy Research Laboratories, Gurgaon, India), pentylene-tetrazol (PTZ; Sigma, St. Louis, MO, USA) and picrotoxin (PTX; Sigma, St. Louis, MO, USA). All the drugs were dissolved in 0.9% w/v NaCl. All the doses were chosen as per Williams et al.\textsuperscript{10} and the preliminary studies carried out in the laboratory. Different doses were administered intraperitoneally in a fixed volume of 1 ml/100g body weight 30 min before the animals were subjected to test. All the drugs were administered intraperitoneally except cefazolin which was injected intravenously. In combination studies the pre-determined dose of cefazolin was administered 5 min before challenging the animals to the sub-convulsive doses of either PTZ or PTX.

The experimental protocol consists of various groups viz. Group 1: Cefazolin (500-3000 mg/kg, iv) \textit{per se}; Group 2: pentylene-tetrazol (40-80 mg/kg, ip) \textit{per se}; Group 3: picrotoxin (4-16 mg/kg, ip) \textit{per se}; Group 4: cefazolin (500 mg/kg, iv) \times pentylene-tetrazol (40 or 60 mg/kg, ip); Group 5: cefazolin (500 mg/kg, iv) \times picrotoxin (4 or 8 mg/kg, ip); Group 6: cefazolin (1000 mg/kg, iv) \times pentylene-tetrazol (40 or 60 mg/kg, ip); Group 7: cefazolin (1000 mg/kg, iv) \times picrotoxin (4 or 8 mg/kg, ip); Group 8: diazepam (0.5, 1 or 2 mg/kg, ip) \times cefazolin (3000 mg/kg, iv)

Experimental protocol

\textit{Cefazolin-induced convulsions in mice} — Cefazolin at different doses (500-3000 mg/kg) was administered intravenously through the tail-vein in mice. Animals were placed in plexi-glass chamber and observed for a period of 30 min post cefazolin administration. The parameters noted were mean onset time of jerks, clonus, extensor, and % mortality due to cefazolin.

\textit{Pentylene-tetrazol- and picrotoxin-induced convulsions in mice} — Pentylene-tetrazol (40-80 mg/kg) and picrotoxin (4-16 mg/kg) were injected intraperitoneally to mice. Animals were placed in plexi-glass chamber and observed for a period of 30 min post PTZ or PTX administration. The parameters noted were mean onset time of jerks, clonus, extensor, and % mortality due to PTZ.\textsuperscript{21,22}

Statistical analysis — One specific group of mice was assigned to one specific drug treatment condition and each group comprised 6–10 mice. All the values were expressed as mean ±SE. The data were analyzed by using One way Analysis of Variance (ANOVA) followed by Tukey’s test. In all tests, the criterion for statistical significance was \(P < 0.05\).

Results

\textit{Convulsive potential of different doses of cefazolin (500-3000 mg/kg, iv) in mice} — Cefazolin, when administered intravenously at doses of 3000 mg/kg produced wild running, jumping and rolling movements followed by severe convulsions in mice (showed all the phases of convulsions viz. jerks, clonus and extensor and finally death). Lower doses (500, 1000 or 2000 mg/kg, iv) failed to induce any behavioral abnormality or signs of convulsions in mice (Fig. 1a-d). Although 2000 mg/kg, iv cefazolin failed to produce any convulsive behaviour or mortality in mice in the present study, a dose of 500 or 1000 mg/kg, iv was selected as sub-effective doses of cefazolin for further pro-convulsant studies.

\textit{Effect of pretreatment of cefazolin (500-2000 mg/kg, iv) with sub-convulsive/minimum doses of pentylene-tetrazol or picrotoxin in mice} — Pentylene-tetrazol (80 mg/kg, ip) or picrotoxin (16 mg/kg, ip) produced severe tonic-clonic convulsions in mice followed by 100 % mortality. PTZ (60 mg/kg, ip) or PTX (8 mg/kg, ip) induced mild tonic-clonic convulsions with decreased mortality while still lower doses of PTZ (40 mg/kg, ip) or PTX (4 mg/kg, ip) failed to produce any mortality or observable behavioral changes (Fig.1a-d). For subsequent pro-convulsant studies, a sub-convulsive/ minimum dose of PTZ (40 or 60 mg/kg, ip) or PTX (4 or 8 mg/kg, ip) was selected to observe the convulsive potential of cefazolin.

The sub-convulsive dose of cefazolin (500 or 1000 mg/kg, iv) when injected 5 min before the administration of sub-convulsive/minimum doses of PTZ (40 or 60 mg/kg, ip) or PTX (4 or 8 mg/kg, ip), enhanced the convulant ability of the later drugs as shown by decreased onset time of jerks, clonus and extensor phase (Figs 2a-c and 3a-c). There was significant increase in mortality in the combination group as compared to either drug \textit{per se} (Figs. 2d and 3d).

\textit{Effect of diazepam (0.5-2 mg/kg, ip) on cefazolin (3000 mg/kg, iv)-induced convulsions in mice} — Cefazolin (3000 mg/kg, iv) induced behavioral excitation such as wild running, jumping and rolling movements (data not shown), severe convulsions and
death were inhibited by diazepam (0.5-2 mg/kg, ip) (Fig. 4a-d).

Discussion

Cefazolin, a first generation parenteral cephalosporin\textsuperscript{1,2} belongs to β-lactam class of antibiotics with a wide spread use for the treatment of various bacterial infections\textsuperscript{3,4}. Systemic administration of various β-lactam antibiotics produces seizures in various animal models of epilepsy\textsuperscript{9,23,24}. The epileptogenic properties of these antibiotics, like their antimicrobial properties, are dependent on the integrity of the β-lactam bond\textsuperscript{25}. The tetrazole nucleus at position 7 of the cefazolin shows a marked chemical similarity to pentylenetetrazol\textsuperscript{26} and
therefore, the present results with cefazolin confirm the implications on its role in producing convulsions. Various studies provide strong evidences that cefazolin has similar intrinsic convulsive activity to other penicillins\textsuperscript{27-29}. While elucidating the potential convulsive risk of this antibiotic in clinical use, emphasis should be given to its convulsive activity in experimental animal models. Pentylenetetrazol or picrotoxin-induced seizures are well accepted animal model of epilepsy involving GABA\textsubscript{A} antagonistic property\textsuperscript{22,30-32}. Pentylenetetrazol or picrotoxin interact with distinct domains of the GABA\textsubscript{A} receptors\textsuperscript{33} and block GABA-induced Cl\textsuperscript{-} currents\textsuperscript{34-37}. Cefazolin showed binding affinity to GABA\textsubscript{A} receptors in concentration-dependent and competitive manners\textsuperscript{38} and also inhibited [$^3$H] GABA binding to mouse synaptic membranes and induced convulsions in a concentration dependent manner\textsuperscript{24}.

In the present study, cefazolin \textit{per se} at higher dose demonstrated all the parameters of convulsions in mice supporting the clinical reports regarding the ability of cephalosporins to produce seizures in hospitalized patients receiving continuous iv infusion of this class of drugs\textsuperscript{39,40}.  

Fig. 3 — Pro-convulsant action of cefazolin combined with PTZ or PTX on the mean onset time of (a) jerks, (b) clonus, (c) extensor and (d) mortality in mice. In the combination study, cefazolin (Cef, 1000 mg/kg, iv) was administered 5 min before challenging the animals to different doses of either PTZ (40 or 60 mg/kg, ip) or PTX (4 or 8 mg/kg, ip). $P$ values: $< 0.05$; as compared to *cefazolin treated group; $^\prime$PTZ treated group; and $^\prime$PTX treated group; ANOVA followed by Tukey’s test.

Fig. 4 — Effect of diazepam (0.5-2 mg/kg, ip) on the mean onset time of (a) jerks, (b) clonus, (c) extensor and (d) mortality produced by cefazolin (3000 mg/kg i.v.) in mice. $P$ values: $< 0.05$; as compared to *vehicle treated control group; ANOVA followed by Tukey’s test.
The study used iv route to administer the toxic/convulsive dose of cefazolin in order to improve the bioavailability and thus increasing the amount of the drug penetrating blood brain barrier. At normal concentration cefazolin does not cross the blood brain barrier and so the amount of the drug reaching cerebrospinal fluid (CSF) is less\(^3\) but when it is administered in massive doses through the intravenous route, it is assumed that the drug concentration in CSF is in “instantaneous” distribution equilibrium with that at the site of neurotoxic action, whereas there is an initial disequilibrium between the concentration either in serum or in the whole brain and at the site of action\(^4\). In the present study, cefazolin did not produce any convulsive behaviour at least in normal conscious mice up to 2000 mg/kg, iv (Fig.1a-d). This is in accordance with the fact that its penetration into the brain is quite low in animals\(^2,3\). Cefazolin did not produce signs of toxicity or convulsive behaviour until lethal levels were approached at doses of approximately 2500 to 3333 mg/kg, iv in mice (data not shown). Further, the sub-convulsive doses of cefazolin (500 or 1000 mg/kg i.v.) when combined with the sub-convulsive/minimum doses of either PTZ or PTX, showed an augmented response with these convulsive drugs and this attributed its potential to enhance the effect of various GABA\(_A\) antagonists and to reduce the inhibitory GABAergic neurotransmission. The involvement of N-methyl D-aspartate (NMDA) receptors in cefazolin induced seizures cannot be ruled out but convincing evidences are in favour of its GABA\(_A\) antagonistic property\(^38\).

In the present study, it was shown that cefazolin at higher doses produced convulsions in mice and the subeffective dose of it potentiates the convulsant ability of PTZ or PTX, the GABA\(_A\) antagonists. This effect clearly supports the hypothesis that cefazolin also competitively inhibits GABA-induced Cl\(^-\) currents by binding directly to the receptor and consequently results in the inhibition of GABA\(_A\) receptor-mediated inhibitory response.

Various experimental evidences support the involvement of \(\gamma\)-amino butyric acid (GABA) in seizures. GABA is the principal inhibitory neurotransmitter which attenuates the seizures by binding to GABA receptors involved in activating the inhibitory cascade in different CNS regions and thus plays a ubiquitous role in reducing the excitatory tone. Numerous natural and synthetic compounds bind to different modulatory sites of GABA receptors. Diazepam is a positive modulator of GABA\(_A\) receptors\(^44\) which inhibits the seizure frequency and severity by increasing the GABA\(_A\) receptor mediated Cl\(^-\) conductance\(^32\). In the present study, the convulsant effect of cefazolin was inhibited by diazepam further indicating that cefazolin induced seizures were due to negative GABAergic modulation.

References


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