PHARMACOLOGICAL EFFECTS OF CEFCLIDIN ON THE CARDIOVASCULAR AND AUTONOMIC NERVOUS SYSTEMS

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The effects of cefclidin (CFCL), a novel antibacterial agent, on the cardiovascular and autonomic nervous system were examined using a variety of *in vivo* and *in vitro* models. The effects of cefazolin (CEZ) were also examinated for comparative purposes.

Neither CFCL nor CEZ caused any notable effects on the cardiovascular or autonomic nervous system when administered intravenously to anaesthetised dogs and cats or *in vitro* preparations. Some evidence of a possible transient stimulation of the sympathetic nervous system was seen following high doses of both CFCL and CEZ.

Key words : cefclidin, general pharmacology, cardiovascular, autonomic nerve, respiration.

Introduction

Cefclidin (CFCL) is a new parenteral cephalosporin synthesized by Eisai Co. Ltd, Japan. It has a broad antibacterial spectrum and potent activity against gram-negative bacteria including *Pseudomonas aeruginosa*. CFCL also showed high resistance to hydrolysis by and low affinity for various types of β-lactamases.

This paper describes the results of the pharmacological studies with CFCL in comparison with cefazolin (CEZ). CFCL and CEZ were each evaluated at two dose levels using a battery of standard laboratory tests designed to investigate the effects on the cardiovascular and autonomic nervous systems.

Materials and Methods

1. Materials

1) Animals

Male Wistar rats (203−226 g), male New Zealand White rabbits (2−3 kg), male and female cats (3.45−4.7 kg) and male and female beagle dogs (9.5−11.0 kg) were housed appropriately in air-conditioned temperature and humidity controlled rooms. The lighting schedule was 12 : 12 light/dark.

The animals were fed an appropriate laboratory diet or commercial pet food. Tap water was available *ad libitum* except where indicated.

2) Test compounds

CFCL and CEZ were dissolved in physiological saline solution at different concentrations.

The other test materials used were cefazolin (CEZ) (Fujisawa Pharmaceutical Co. Limited), noradrenaline, adrenaline, acetylcholine, pilocarpine, adenosine diphosphate, 1-1-dimethyl-4-phenyl piperazinium iodide (DMPP) (Sigma Chemical Company) : anaesthetic ether, sodium thiopentone (May Baker Limited) : heparin sodium (Evans Medical Limited) chloralose (BDH Chemical Limited) : collagen (Hormon Chemie) and trisodium citrate (FSA Laboratory Supplies). The materials were dissolved in a medium compatible with the test procedure.

2. Methods

1) Effects on respiration, blood pressure, heart rate, femoral blood flow and electrocardiogram

Three male dogs were used in this experiment. Dogs were anaesthetised with sodium thiopentone

(25mg/kg i.v.) and maintained under α-chloralose (60−80mg/kg i.v.). Respiratory rate and tidal flow were recorded with the aid of a respiratory transducer (Grass PT5A) connected to the cannulated trachea. The blood pressure was recorded by means of a pressure transducer (Bell and Howell) connected to a cannulated femoral artery. Heart rate was determined by integration of pulse waves with a tachometer (Grass Instruments). The blood flow in a femoral artery was recorded with the aid of an electromagnetic flow meter (Carolina). In addition, the ECG (LeadII) was monitored by appropriately positioned limb leads. All recordings were made on a Grass Instruments chart recorder. Drugs were injected into the cephalic vein through an indwelling catheter. The effects of the test compounds on the cardiovascular and respiratory parameters were recorded over a 30 minute observation period. Vehicle and two doses of both CFCL and CEZ were examined in each dog.

2) Effect on hind limb perfusion

One male and two female dogs were used for this experiment.

The dogs were anaesthetised with sodium thiopentone (25mg/kg i.v.) and maintained under α-chloralose (60−80mg/kg). Blood pressure was recorded by means of a pressure transducer (Bell and Howell) connected to a cannulated femoral artery. The trachea was cannulated to allow unrestricted ventilation.

The contralateral femoral artery was bicannulated. Blood from the central end of the artery was pumped via a Watson Marlow flow inducer into the distal end. Perfusion pressure was measured by means of pressure transducer (Bell and Howell) connected to the perfused femoral artery by means of a T connector. The pump was adjusted such that the maximum perfusion pressure was equivalent to the resting systolic blood pressure. The dogs received 25,000 units of heparin prior to the start of the experiment.

Vehicle and two doses of both CFCL and CEZ were examined in each dog. The effects of the test drugs on the hind limb perfusion pressure were recorded over a 30 minute observation period. A minimum dose interval of 30 minutes was used.

3) Isolated perfused rabbit heart

The method used was essentially as described by Langendorff[1]. The heart was removed from four male rabbits. The aorta was cannulated and the coronary circulation was perfused with Krebs solution maintained at 37℃ and aerated with 95% O_2 and 5 % CO_2 by means of a Watson Marlow flow pump. The hearts were perfused at a sufficient rate to provide a perfusion pressure of 20−60 mmHg which was recorded by means of a pressure transducer (Bell and Howell). The force of contraction was measured by means of a Dynamometer UF1 strain gauge attached to the apex of the heart. Heart rate was derived electronically and all parameters were recorded using a Lectromed chart recorder.

4) Effect on contraction of the nictitating membrane and the cardiovascular response to noradrenaline and bilateral carotid occlusion

The methodology was based on that described by Bevan[2].

One male and two female cats were used for this experiment. The cats were anaesthetised with ether and maintained under α-chloralose (60−80mg/kg i.v.). Bipolar electrodes were sited beneath the preganglionic trunk of the left superior cervical nerve and a second beneath the post-ganglionic trunk of the right superior cervical nerve. To elicit a contraction of the nictitating membrane the appropriate nerve was stimulated with rectangular pulses of supramaximal voltage (50 Hz, 1 ms, 10 s Grass S88). The contraction of the nictitating membrane was recorded with the aid of isotonic transducers (Devices).

Responses of blood pressure and heart rate to intravenous administration of 1μg/kg noradrenaline and to bilateral carotid occlusion (BCO) were also recorded by means of a pressure transducer (Bell and Howell) connected to a cannulated femoral artery. All parameters were recorded using a Lectromed chart recorder. The test drugs and noradrenaline were injected into a cephalic vein through an indwelling catheter. The effects of the test compounds on the responses to pre- and postgangilionic nerve stimulation and to BCO and noradrenaline were recorded during each 30 minute

observation period. Vehicle and two doses of both CFCL and CEZ were examined in each cat.

5) Effect on the cardiovascular response to vagal stimulation, acetylcholine and DMPP.

Three male cats were used for this experiment. Cats were anaesthetised with ether and maintained with α-chloralose (60−80mg/kg) administered intravenously. A femoral artery and cephalic vein were cannulated to facilitate measurement of blood pressure and the administration of anaesthetic, agonists and test compounds respectively. The femoral artery cannula was connected to a heparin/saline (250 IU/ml)-filled Bell and Howell pressure transducer coupled to a Grass Model 7E polygraph. The heart rate was derived electronically from the blood pressure signal.

The trachea was catheterised. Both cervical vagi were separated from connective tissure and severed at their cardiac ends. The central ends were stimulated by pairs of bipolar electrodes. The nerves were stimulated at 30 Hz, pulse width 5 ms, 6 7 volts for 30 seconds to elicit reproducible changes in blood pressure and heart rate.

Following equilibration, thirty minute cycles consisting of vagal stimulation, injections of 1μg/kg acetylcholine and 10μg/kg DMPP at approximately 10 minute intervals were repeated twice. Vehicle (5 ml/kg 0.9% saline) was administered intravenously, over two minutes, commencing 5 minutes after the second dose of DMPP and 5 minutes before a vagal stimulation which initiated a third cycle. At the same point in each subsequent cycle a dose of one of the test articles was administered. Extra cycles were included between doses if it was judged that a change had occurred which was not within the normal range.

6) Pilocarpine antagonism test

The method used is based on that described by Janssen and Niemegeers[3].

Groups of ten male rats were used for this experiment. Ten minutes following intravenous administration of the test drug, each rat received a rapid intravenous injection of 80mg/kg of pilocarpine hydrochloride.

The pupil diameter of each eye was measured immediately before and five munutes following pilocarpine administration using a stereomicroscope with a scaled eye-piece.

The intensity of salivation and lacrimation (or chromodacryorrhoea) was scored on a 0 3 scale between 5 and 10 minutes following pilocarpine injection.

7) Haemolytic effect

Three male rabbits were used for this experiment. Five ml of blood was taken from a marginal ear vein of each rabbit and placed into tubes containing EDTA anticoagulant. Following centrifugation the red cells were washed three times by repeated resuspension in saline and centrifugation. Following the final wash a 3% suspenision of the red cells was prepared in isotonic saline.

The appropriate concentration of the test drug was then added to the red cell suspension and incubated at 37℃ for 2 hours.. Following centrifugation, the absorbance of the supernatant was measured in an 'EEL' spectophotometer at 540 nm.

The percentage haemolysis was determined for each sample.

8) Effect on blood coagulation

Groups of 10 male rats were used for this experiment. Five minutes after intravenous administration of the test compounds the rats were bled by tail puncture and the whole-blood clotting time was determined immediately using the method of Dale and Laidlaw[4]. The rats were then anaesthetised with ether and a blood sample removed from the orbital sinus for determination of prothrombin time and activated partial thromboplastin time by the method of Quick[5] and Proctor and Rapaport[6] respectively.

9) Effect on pupil diameter

Groups of 5 male rabbits were used for this experiment which was conducted in a room with low light intensity. The pupil diameter of both eyes was assessed subjectively one hour before and 1/4, 1/2, 1, 2 and 4 hours after intravenous administration of test drugs.

10) Effect on platelet aggregation

The method used was based on that described by Born[7].

A pooled platelet-rich plasma sample (PRP) was

prepared using blood from healthy human volunteers of either sex. Platelet-rich plasma was prepared by centrifuging citrated blood samples at 1,000 rpm for 10 minutes and pooling the resultant plasma.

The concentration of platelets was adjusted to 300,000 per mm^3 using pooled platelet-poor plasma obtained from the same donors.

Appropriate concentrations of the test compounds were added to aliquots of the PRP and incubated for 15 minutes at 37℃.

Aggregation was then induced by addition of ADP (2μg/ml), collagen (10μg/ml), or adrenaline (5×10^{-6}M) and measured using a Bryston aggregometer and the traces were recorded on a Servogor recorder.

Results

1) Effects on respiration, blood pressure, heart rate, femoral blood flow and electrocardiogram (Figures 1-3)

CFCL and CEZ were examined at doses of 300 and 1,000mg/kg. CFCL tended to temporarily increase blood pressure at a dose of 300mg/kg but had variable effects after 1,000mg/kg. CEZ also tended to cause temporary dose-related increases in blood pressure. Heart rate tended to be reduced by CFCL at both doses. The effects were small and transient. CEZ tended to cause temporary increases in heart rate.

An effect on respiration was seen after both compounds characterised by a dose-related reduction in tidal volume and increase in respiration rate. The magnitude of responses after each compound were comparable however after 1,000mg/kg CEZ the effect were sustained.

An increase in femoral arterial blood flow was observed after both CFCL and CEZ. In conjunction with these increases, femoral resistance was reduced. Recovery from these changes occurred within 30 minutes.

The electrocardiogram was not affected by either dose level of CFCL or CEZ

2) Effect on hind limb perfusion (Figure 4)

CFCL and CEZ were administered intravenously

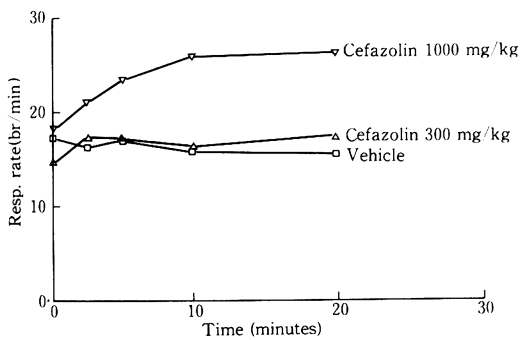

Fig. 1-a. Effect of intravenous administration of cefazolin on respiration rate in the anaesthetised dog

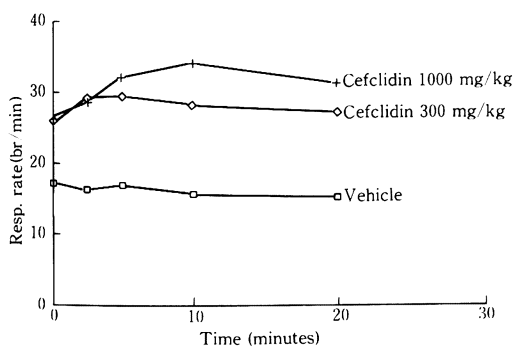

Fig. 1-b. Effect of intravenous administration of cefclidin on respiration rate in the anaesthetised dog

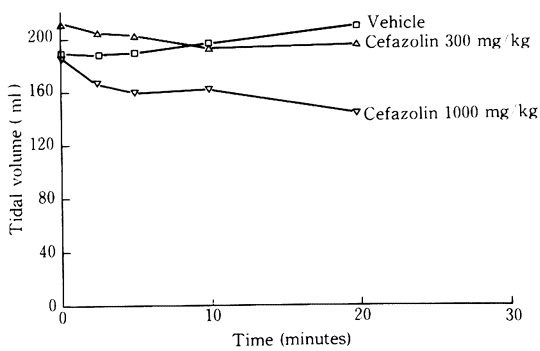

Fig. 2-a. Effect of intravenous administration of cefazolin on tidal volume in the anaesthetised dog

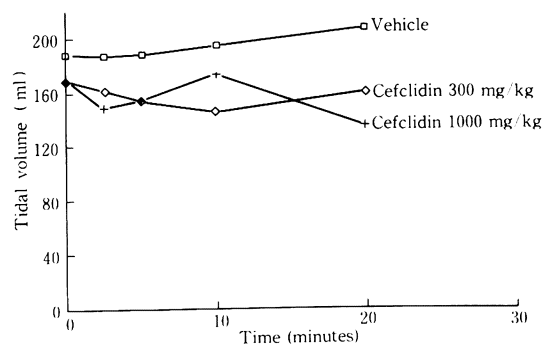

Fig. 2-b. Effect of intravenous administration of cefclidin on tidal volume in the anaesthetised dog

at 300 and 1,000mg/kg.

CFCL at 300mg/kg reduced hind limb perfusion pressure and recovery was complete within fifteen minutes. CFCL also caused a reduction in perfusion pressure at a dose of 1,000mg/kg. CFCL increased blood pressure and decreased heart rate.

CEZ increased hind limb perfusion pressure at both doses. Reductions in hind limb perfusion pressure were seen in two dogs after 300mg/kg and all dogs after 1,000mg/kg.

All dogs exhibited tremor after 1,000mg/kg CEZ which gradually diminished over the course of the experiment.

3) Isolated perfused rabbit heart

CFCL and CEZ were perfused at concentrations of 2×10^{-5} and 2×10^{-4}M. This range of concentrations had no effect on perfusion pressure, force of contraction or heart rate.

4) Effect on contraction of the nictitating membrane and the cardiovascular response to noradrenaline and bilateral carotid occlusion

Administration of the lowest dose (300mg/kg) of either CFCL or CEZ failed to modify the cardiovascular responses to noradrenaline or bilateral carotid occlusion. Contractions of the nictitating membrane to pre- and post-ganglionic stimulation were similarly unaffected.

CFCL and CEZ (1,000mg/kg) had no effect on the responses to noradrenaline. In general the responses of the nictitating membrane to pre- and post-ganglionic stimulation were similarly unaffected although one cat showed signs of a weak ganglionic blockade following CEZ. Both CFCL and CEZ evoked a slight inhibition of the response to bilateral carotid occlusion.

5) Effect on the cardiovascular response to vagal stimulation, acetylcholine and DMPP.

CFCL and CEZ were administered intravenously at 300 and 1,000mg/kg.

The responses of blood pressure and heart rate to vagal stimulation, acetylcholine or DMPP were unaffected by any of the treatments.

CEZ (1,000mg/kg) induced mild muscle tremor in one animal.

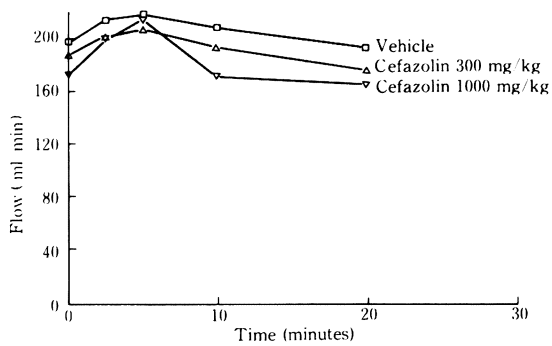

Fig. 3-a. Effect of intravenous administration of cefazolin on femoral blood flow in the anaesthetised dog

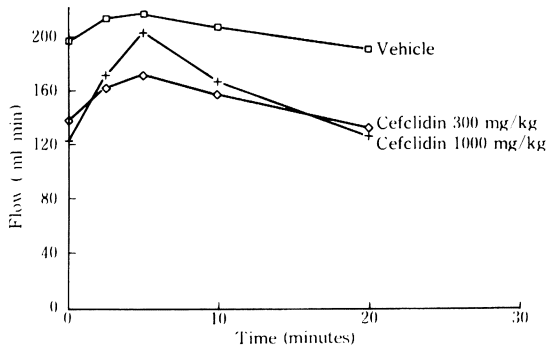

Fig. 3-b. Effect of intravenous administration of cefclidin on femoral flow in the anaesthetised dog

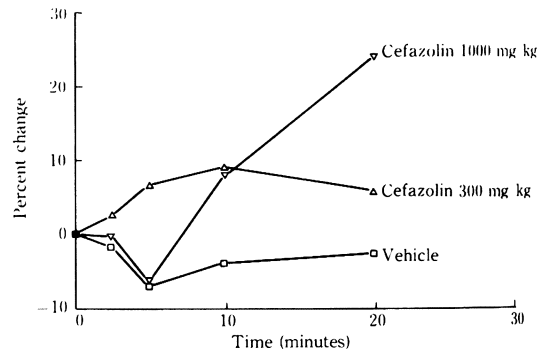

Fig. 4-a. Effect of intravenous administration of cefazolin on hind-limb perfusion pressure in the anaesthetised dog

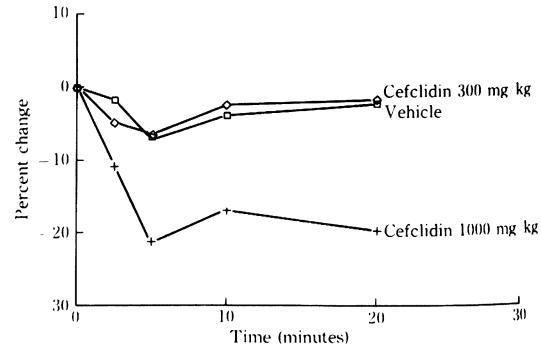

Fig. 4-b. Effect of intravenous administration of cefclidin on hind-limb perfusion pressure in the anaesthetised dog

6) Pilocarpine-antagonism test (Table 1)

The possible anticholinergic effect of CFCL and CEZ were examined following intravenous doses of 1,000 and 2,000mg/kg.

CFCL at the highest dose (2,000mg/kg) caused a marked inhibition of pilocarpine-induced lacrimation. A weak decrease in salivation was also observed. General signs of toxicity, however, were also noted at this dose level.

CEZ caused a weak potentiation of pilocarpine-induced cholinergic effects.

7) Haemolytic effect

At the concentrations examined ($2\times10^{-5}$and 2×10^{-4}M) both CFCL and CEZ were devoid of any noteworthy haemolytic effects.

8) Effect on blood coagulation

CFCL and CEZ at doses of 300 and 1,000mg/kg intravenously failed to cause any overt effects on blood coagulation parameters.

9) Effect on pupil diameter

Intravenous administration of CFCL and CEZ at doses of 300 and 1,000mg/kg produced no significant effect on pupil diameter in the rabbit.

10) Effect on platelet aggregation (Table 2)

The effects of CFCL and CEZ (1×10^{-4}M and 1×10^{-5}M) on ADP, collagen and adrenaline-induced human platelet aggregation were examined.

The most notable effects of CFCL was upon adrenaline-induced platelet aggregation where a potentiation value of 30% was recorded at a concentration of 1×10^{-4}M.

The most notable effect of CEZ was upon collagen-induced platelet aggregation where a potentiation value of 19% was recorded at a concentration of 1×10^{-4}M.

No other effects of note were observed for either compound.

Table 1. Effects of intravenous administration of cefclidin and cefazolin on pilocarpine-induced changes in pupil diameter, lacrimation and salivation in the rat

Group	Intravenous treatment	Dose (mg/kg)	Group mean pupil diameter (mm ± SD) before and after pilocarpine		Group mean score for salivation ± SD	Group mean score for lacrimation ± SD
			Before	After		
1	Vehicle (0.9% saline)	–	0.50 ±0.06	2.28 ±0.60	1.90 ±0.74	1.30 ±0.48
2	Cefclidin	1000	0.52 ±0.04	2.50 ±0.51	1.80 ±0.79	1.50 ±0.53
3	Cefclidin	2000	0.49 ±0.14	2.17 ±0.86	1.11 ±1.05	0***
4	Cefazolin	1000	0.48 ±0.10	2.83* ±0.67	1.80 ±1.14	1.40 ±0.70
5	Cefazolin	2000	0.51 ±0.02	2.85** ±0.73	2.40 ±0.52	2.00* ±0.82

Statistical significance of difference from control group using analysis of variance (Student's 't' distribution):
* : $p<0.05$ ** : $p<0.01$ *** : $p<0.001$

Table 2. Effects of cefclidin and cefazolin on *in vitro* human platelet aggregation induced by ADP, collagen and adrenaline

Treatment	Concentration (M)	Percentage inhibition of aggregation induced by :			Percentage potentiation of aggregation induced by :		
		ADP (2μg/ml)	Collagen (10.0μg/ml)	Adrenaline (5.0μmol/l)	ADP (2μg/ml)	Collagen (10.0μg/ml)	Adrenaline (5.0/μmol/l)
Cefclidin	1×10^{-4}	0	2	0	0	0	30
Cefclidin	1×10^{-5}	0	8	4	12	0	0
Cefazolin	1×10^{-4}	0	0	0	3	19	0
Cefazolin	1×10^{-5}	2	0	0	0	9	9

Discussion

CFCL and CEZ were examined in a variety of *in vivo* and *in vitro* models selected to investigate effects on the cardiovascular and autonomic nervous systems.

In the anaesthetised dog preparation CFCL and CEZ showed few noteworthy effects on systemic blood pressure, heart rate or ECG. Both compounds, however, evoked a transient increase in mean femoral arterial blood flow. At the low dose the effect of both CEZ and CFCL was comparable to that seen with vehicle. The effect, however became more marked at the higher dose.

The apparent vasodilation caused by CFCL and CEZ may not have been due to a local action. In a separate anaesthetised dog experiment, where perfusion of the hind limb was maintained constant, intravenous administration of CFCL evoked a decrease in perfusion pressure suggestive of a sympathetically mediated action. CEZ similarly induced a reduction in perfusion pressure, which was however subsequently followed by a vasoconstriction.
Further support for a transient stimulation of the sympathetic nervous system was the increase in respiration rate with concurrent decrease in tidal volume seen in the dog after drug administration.
In the rabbit, CFCL at the highest dose (2,000 mg/kg) inhibited pilocarpine-induced lacrimation. Whilst general signs of toxicity were also observed at this very high dose, this effect could also be attributed to sympathetic stimulation rather than an inhibition of the cholinergic post-ganglionic parasympathetic receptors. The effect would seem to be associated only with very high dose levels as no evidence of an effect on pupil diameter was observed in rabbits receiving doses of 1,000 mg/kg of CFCL or CEZ.

Experiments in theanaesthetised cat failed to reveal any effects on the efferent parasympathetic pathway by either CFCL or CEZ.

Intravenous administration of the two drugs failed to induce any remarkable effects on blood coagulation parameters or to disrupt the structure of erythrocytes. The apparent potentiation of platelet aggregation induced by adrenaline or collagen by the highest concentration is unlikely to be of any biological significance.

It may be concluded that neither CFCL nor CEZ induced any notable effects on the cardiovascular and autonomic nervous system altough a slight transient stimulation of the sympathetic nervous system may be associated with intravenous administration of very high doses.

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Cefclidin一般薬理作用：呼吸・循環・自律神経系への影響

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新規の抗生剤，cefclidin（CFCL）の呼吸・心脈管・自律神経系に対する影響について，cefazolin（CEZ）と比較検討した。

麻酔ビーグル犬において，CFCLは300mg/kgおよび1,000mg/kgで，用量依存的に呼吸数と分時換気量が増加した。但し1回換気量は変化しなかった。血圧は一過性に軽度上昇し，それに伴う心拍数の減少がみられた。心電図には影響しなかった。また後肢還流圧の一過性低下が認められた。さらにnoradrenaline,acetylcholine,DMPP,両側頚動脈閉塞，迷走神経刺激時の心脈管反応，あるいは神経節刺激時の瞬膜収縮に影響しなかった。一方，CEZについても同様の変化が認められ，CFCLより比較的持続的であった。その他，特に麻酔動物においても振せんが観察された。

CFCLは2,000mg/kgでpilocarpineによる流涙反応を抑制した。その他，血液系に対し，1 x10^{-4}Mでadrenaline惹起血小板凝集を軽度増強した以外，血液凝固，溶血作用などへの影響はなかった。

以上，CFCLは心脈管あるいは自律神経系に対して，問題となる影響はないと思われる。

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