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Antinociceptive action of Icon®, a pyrethroid insecticide on pregnant rats

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Abstract

Icon® is a type II synthetic pyrethroid insecticide based on active ingredient Lambda cyhalothrin (10% w/w). In a recent study it was evident that this insecticide although induced marked signs of stress and other overt signs of toxicity in pregnant rats it never provoked aversive behaviours: suggestive of antinociceptive activity. Hence this study was conducted to investigate the possible antinociceptive potential of Icon® on pregnant rats. Different doses of Icon® 0, 63, 83 or 125 mg/ kg body weight/day (active ingredient 0, 6.3, 8.3, 12.5 mg / kg body weight/day) was orally administered to pregnant (early, mid and late) rats for seven consecutive days and antinociception potential was determined using the hot plate technique. The results showed a marked antinocicpetive activity (in terms of prolongation of reaction time) of Icon in pregnant rats. It is concluded that the antinociception is mediated by both specific (neuronal) and non specific (stress and food inhibition) mechanisms.

Key words: Antinociception, Icon®, Lambda-cyhalothrine, insecticide, pyrethroid, pregnancy

1. Introduction

Icon®, a water miscible type II synthetic pyrethroid insecticide based on active ingredient Lambda cyhalothrin (IPCS-99 Cyhalothrin, 1990) (10% w/w: information on inert fillers, adjuvants, excipients, wetting agents and purity are not available) has been recently introduced to Sri Lanka as an

adulticidal indoor spray against malaria vector mosquitoes (Manuweera G., Registar of pesticides, Sri Lanka, personnel communication). Since several insecticides are known to interrupt mammalian reproduction and pregnancy (Watterson, 1999, Jayatunga *et al.,* 1998a and Jayatunga *et al.,* 1998 b) we investigated the antireproductive effects of Icon® on pregnant rats (Ratnasooriya *et al,* 2003,and Ratnasooriya *et al.*,2003, submitted). During these investigations we noted marked but reversible overt clinical signs of toxicity on treated rats. However, we never observed any aversive behaviours: in terms of self biting and scratching, shaking of body, liking of tail or and paws, intense self-grooming behaviour, bouts of spontaneous agitation and vocalization with Icon® (Ratnasooriya *et al.*,2003). Lack of these effects is suggestive of an antinociceptive action. Additionally, in humans, exposure to Lambda cyhalothrin reported to result in tingling, burning and numbness, features of neuropathy (IPCS-99 Cyhalothrin, 1990). Further, analgesic activity has been reported with methamidophos, an organophophorus insecticide, (Peiris, *et al.,* 1994) which was commercially used in Sri Lanka but banded now (Manuweera G., Registrar of Pesticides, Sri Lanka, personnel communication). These prompted us to investigate experimentally, the antinociceptive effects of Icon® on pregnant rats with different stages of gestation using the same treatment regimes used previously (Ratnasooriya *et al.,* 2003 and Ratnasooriya *et al*, 2004, in press) which was the aim of this study.

2. Materials and Methods

Animals

Healthy adult cross-bred albino female and male (200 -250 g) rats from the colony at the Department of Zoology, University of Colombo, Sri Lanka, were used. These animals were kept singly in plastic cages, under standardized animal house conditions (temperature: 28°C-30°C; natural photoperiod: approximately 12 h light and 12 h dark; relative humidity (50 -55%). Rats were fed with pelleted food (Master Feeds Lanka Ltd., Bambalapitiya, Sri Lanka) and tap water *ad libitum*

Icon® preparation

Icon® was obtained from Anti-Malaria Campaign, Narahenpita, Sri Lanka. Three desired doses [63 (low), 83 (mid) and 125 (high) mg/kg/day of Icon®; (containing active ingredient Lambda cyhalothrin 6.3, 8.3,12.5 mg/kg/day respectively)] in lml aliquots were prepared by mixing Icon® in distilled water (DW). These doses are comparable to what has been used previously

to investigate the effects of Icon on pregnant rats (Ratnasooriya *et al.*, 2003, in press). The reported oral non-observed effect of Lambda cyhalothrin is 50 mg/kg (IPCS-99 Cyhalothrin, 1990).

Icon administration

Pro-oestrus rats were selected from regularly cycling healthy adult females through examination of vaginal smears using 0.9% w/v normal saline under an Olympus light microscope (Olympus Optical Company Ltd, Tokyo, Japan) at X 40 magnification. Positive pro-oestrus females were then individually paired (15.00 h - 17.00 h) over night with male rats of proven fertility. Next morning (08.00 h - 09.00 h) vaginal smears were taken using normal saline and the insemination was confirmed by the presence of spermatozoa in the vaginal smear. The number of spermatozoa was estimated using a haemocytometer (Improved Neubauer, Weber Scientific International Ltd. England) under Olympus phase contrast microscope (Olympus optical company Ltd., Tokyo, Japan) at X 400 magnification. If the number of spermatozoa was > 5 X 10^6 ml^{-1}', then those females were considered as the successfully mated females and this day was designated as day 1 of pregnancy. One hundred forty four successfully mated day 1 pregnant rats were selected assigned into 3 groups (G-1, G-2 and G-3). Animals of each group was then subdivided into 4 treatment groups T1 - control (Distilled water), T2 - low dose (63 mg/kg/day of Icon®), T3 - mid dose (83 mg/kg/day of Icon®), and T4 - high dose (125 mg/kg/day of Icon®). Twelve animals were used in each treatment. Icon® or vehicle (DW) was orally administered by gastric intubation between 09.00 -10.00 h for seven consecutive; G-1 from day 17 of pregnancy (early), G-2 from day 8 -14 of pregnancy (mid), G-3 from day 15 -21 of pregnancy (late).

Adverse effects

Following every dosing, cage side observations were made on each rat continuously for 3 - 5 h for mortality, overt signs of toxicity (salivation, lacrymation, convulsions, ataxia, yellowing and loss of fur), stress (exophthalmia, erection of fur), lethargy (reduction of spontaneous walking movements, climbing in cage, cleaning of fur) recumbence, aversive behaviours (self biting and scratching, shaking of body, liking of tail or and paws, intense self-grooming behaviour, bouts of spontaneous agitation and vocalization). In addition, the treated rats were closely observed for the presence of Straub's tail reaction (the tail was held rigid and erected across the back of the animal in a S - shaped curve) and CNS stimulating behaviours (spontaneous agitation, enhanced locomotory activity).

Determination of analgesic activity

About 7 h after final dosing of Icon® or vehicle on final day of each treatment, the rats were singly introduced into the hot plate analgesia meter (Model MK-350A, Muromachi Kikai Company Ltd., Tokyo, Japan), which was at 50^{0}C. Then the reaction time (the time taken to lick the rear foot) of each rat was measured in seconds.

Statistical analysis

Data is expressed as mean ± standard error of mean (SEM). Statistical analysis was made using Mann - Whitney U-test. $P < 0.05$ was considered as statistically significant.

3. Results

Four rats in the early pregnancy group and two rats in the late pregnancy group treated with highest dose of Icon® died during the study period. Further, treated rats did not exhibit aversive behaviours, Straub's tail reaction or CNS stimulating behaviours. Of the toxic signs monitored ataxia, salivation, changes in colour of fur, vaginal bleeding and diarrhoea were evident (Table 1). Further, exophthalmia and pilo erection was evident as stress signs. The adverse effects appeared to be dose - related.

The results obtained with the hot plate test are summarized in Table 2. During mid and late pregnancy, only mid dose (mid pregnancy by 152%; late pregnancy by 214 %) and high dose (mid pregnancy by 176 %; late pregnancy by 246 %) of Icon® significantly ($p < 0.05$) prolonged the reaction time. In contrast, during early pregnancy only the highest dose was abled to increase the reaction time (by 91%).The longest prolongation of the reaction time was evident with the highest dose during late pregnancy.

4. Discussion

This study, examined the antinociception potential of Icon® using hot plate analgesimetric test. This test is sensitive, reliable and widely used in the evaluation of potential analgesic agents (Langerman, *et al.*, 1995). The antinociception action was determined at the same time of each day to minimize diurnal effects: diurnal related variations in pain sensation and relationship to analgesic drugs are reported (Perisan, *et al.*, 2000). The results show that Icon® induced antinociception activity (in terms of prolongation of reaction time) in female rats at early, mid and late pregnancies. The antinociception effect appears to be dose related indicating a causal relationship and possible receptor mediation. Further, the antinociception

potential of Icon® was highest during late pregnancy which could be linked to changes in oestrogen and progesterone levels (Medina, *el al.,* 1993). Interestingly, pyrethroids are reported to act as endocrine disruptors (Garey, *et al.,* 1998) and Icon® to have antiprogestogenic activities (Ratnasooriya *et al.,* 2003, in press) which could change the oestrogen progestrogen balance. In addition, gender and pregnancy related differences with the efficiency of some analgesic drugs are also reported (Kavaliners, *et al.,* 1993).

Icon® induced antinociception is likely to be mediated centrally at the supraspinal level. Further, it may be effective against phasic non-inflammatory type of pain: the hot plate test predominantly measures supraspinally organized responses (Langerman, *et al.,* 1995) and is sensitive to phasic and non inflammatory pains (Langerman, *et al,* 1995). Since Icon® did not provoke Straub's tail reaction and CNS stimulating behaviours, mediation of opioid mechanisms seems unlikely although we cannot exclude the possibility of release of endogenous opioid peptides which are natural analgesics (Bowman, *et al.,* 1975). On the other hand, Icon® being a type II pyrethroid can act on sodium channels in neurolemma to disrupt proper functioning of neurons in the CNS to induce antinociception: (IPCS-99 Cyhalothrin, 1990) as evident in this study: at lower doses pyrethroids cause stable repetitive firing of neurons and at high doses result in depolarization and blockage of nerve conduction (IPCS-99 Cyhalothrin, 1990). Alternatively, Icon® may raise the nociceptive threshold and thereby confer antinociception: pyrethroids are known to increase spontaneous release of GABA (Clark, 1997) which hypopolarize nerve membranes (Bowman, *et al.,* 1975). Further, some analgesics increase nociceptive threshold (Bowman, *et al.,* 1975). However, possibility exists for additional mechanisms to be also operative in inducing antinocicption by Icon in this study. External signs of stress (marked exophthalmia) were evident in Icon® treated rats. Stress induces analgesia (Badio, *et al.,* 1995) and as such may play an important role in producing antinocicpetion here. In rats, food deprivation causes antinocicpetion (McGivern, *et al.,* 1979). We have previously shown that Icon® inhibit food intake in rats (Ratnasooriya, *et al.,* 2003, in press). Thus, it may be possible that such a mechanism has also contributed, at least partly, to the Icon® - induced antinociception.

In conclusion, this study shows that pyrethroid insecticide Icon® induce antinociception in pregnant rats. However, it remains to be seen whether the antinociception is mediated by active ingredient Lambda cyhalothrin or by the inert ingredients or by the contribution of both active and inert ingredients.

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Table 1: Number of rats (n=12/group) displaying overt clinical signs of toxicity after oral administration of Icon® or vehicle during different stages of pregnancy

Parameter monitored	Early pregnancy				Mid pregnancy				Late pregnancy			
	control DW	Treated Icon®mg/kg bodywt./day			control DW	Icon® Treated mg/kg bodywt./day			Control DW	Treated Icon® mg/kg/bodywt./day		
		63	83	125		63	83	125		63	83	125
Mortality	0	0	0	4	0	0	0	0	0	0	0	2
Ataxia	0	1	4	12	0	1	1	6	0	1	4	10
Tremors	0	0	0	0	0	0	0	0	0	0	0	0
Convulsion	0	0	0	0	0	0	0	0	0	0	0	0
Pilo erection	0	0	0	0	0	0	0	2	0	0	0	3
Exophthalmia	0	0	2	4	0	0	0	2	0	0	0	3
Salviation	0	12 (around mouth)	12 (around neck)	12 (Whole body)	0	3 (around mouth)	12 (around neck)	12 (whole body)	0	1 (around mouth)	9 (around neck)	10 (whole body)
Lacrymation	0	0	0	0	0	0	0	0	0	0	0	0
Coughing	0	0	0	0	0	0	0	0	0	0	0	0
Change in colour of fur	0	0	0	0	0	0	0	0	0	0	0	0
Change in colour of urine	0	0	0	0	0	0	1 (dark yellow)	6 (dark yellow)	0	0	3 (dark yellow)	6 (dark yellow)
Vaginal bleeding	0	0	0	0	0	1	3	9	0	0	0	0
Diarrhoea	0	0	2	8	0	1	3	9	0	0	4	7

DW - Distilled Water

Table 2: Effect of different doses of Icon® on hot plate reaction time of preganant rats
(Data represented as mean ±SEM. Ranges are given in parenthesis)

Group	Pregnancy period	Control DW	Treated Icon® mg/kg body wt,/day		
		T1	T2 63	T3 83	T4 125
G1	Early (days 1- 7)	10.2 ±0.9 (4.3 -15.8)	9.3 ±1.6 (3.9 - 17.2)	13.6± 1.4 (6.9-21.0)	19.5± 3.6* 7.5- 38.1)
G2	Mid (days 8-14)	9.4 ±0.6 (6.6-14)	15.0±1.9 (7.4-24.4)	23.7 ±3.6*. (11.9-50.0)	25.9± 4.0* (13.5-50)
G3	Late (days 15-21)	9.7±1.1 (5.3-19.4)	14.3±2.2 (6.7-27)	30.5±4.7* (9.6-50)	33.6±5.1* (9.9-50)

As compared with control, * $p<0.05$, ** $p<0.01$ (Mann-Whitney *U*-test)
DW - Distilled Water