Oral ralfinamide suppresses autotomy following hindpaw deafferentation by multiple dorsal rhizotomies, a rat model of CNS-mediated spontaneous neuropathic pain

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Abstract

Background and aims: L3-6 dorsal rhizotomy (DR) isolates the CNS from the periphery, triggering in rats hindpaw autotomy. Since this spontaneous neuropathic pain-related behaviour is driven by pathophysiological mechanisms in the CNS, we tested whether Ralfinamide (Newron Pharmaceuticals; an analgesic in clinical Phase II/III for neuropathic low back pain) has analgesic CNS targets. Pregabalin was included as a reference compound. ***Methods:*** Male SD rats (N=75, 8-12/group) underwent L3-6 DRs. Autotomy was scored on days 0-63 postoperatively using an accepted scale. Drug effects were evaluated against groups receiving the vehicle or nothing. Preemptive effects were tested by orally administering ralfinamide (80mg/kg, b.i.d.) or pregabalin (10mg/kg, b.i.d.) for 7 days preoperatively. Palliative effects were tested by orally administering ralfinamide (60mg/kg, b.i.d.) or pregabalin (15mg/kg, b.i.d.) for 42 days postoperatively. These doses produce in rats plasma levels comparable to those found in patients administered clinically-relevant doses. ***Results:*** Preemptive effects: Compared to the vehicle, both ralfinamide and pregabalin administered preoperatively delayed autotomy onset (P<0.05), but only ralfinamide suppressed autotomy scores up to day 63 (P<0.01). Palliative effects: Ralfinamide, but not pregabalin, delayed autotomy onset (P<0.01), suppressed its scores during the treatment period of 42 days (P<0.05), and maintained lower scores for 21 days after cessation of drug administration (P<0.05). ***Conclusions***: Ralfinamide, more potently than pregabalin, produced lasting analgesia in the DR model, by affecting the CNS, possibly via its known modulation of sodium channels, glutamate release and NMDAr activity. Neuroprotection and reduced glial activation cannot be excluded as possible mechanisms.

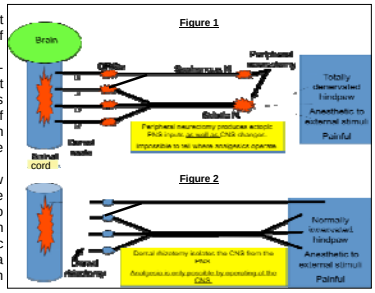
Introduction

Recently we reported in the journal Pain that oral administration of ralfinamide had analgesic effects on spontaneous neuropathic pain behavior (i.e., autotomy) in the neuroma model in the rat. This effect was observed when oral ralfinamide was administered preoperatively ("preemptive analgesia") or post-operatively ("palliative analgesia") (Zhang et al., 2008).

In a complementary study we showed that this effect could result, in part, from a dose-dependent suppression of ectopic firing in injured primary afferents (SfN, 2006). However, since previous reports indicated that the pain-related behavior is this model is driven by ectopic input sources in the periphery and abnormal processing of this input in the CNS (red starts in Fig. 1), suppression of autotomy by systemic treatment cannot distinguish whether the target of this analgesic effect was in the periphery or the CNS.

In contrast to peripheral neurectomy, hindpaw deafferentation by multiple dorsal rhizotomies isolates the CNS from afferent inputs from the hindpaw. This also results in autotomy, but in this case the behavior is driven by input sources within the CNS. Therefore, any analgesic effects seen following systemic administration of a candidate drug must be due to its effect on targets within the CNS.

Figure 1

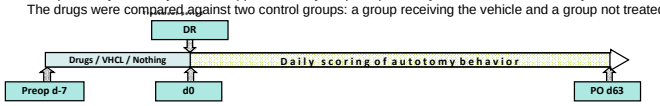


Aims of the study

➢ To compare the analgesic efficacy of ralfinamide and pregabalin on autotomy following total hindpaw deafferentation by multiple dorsal rhizotomies. Pregabalin was selected as a reference drug due to its current status as the leading analgesic drug for neuropathic pain.

➢Two administration modes were tested:

❖Preoperatively for 7 days, then stopped until day 63 postoperatively, the end of the autotomy observation period. The drugs were compared against two control groups: a group receiving the vehicle and a group not treated at all.



❖Postoperatively for 42 days, then stopped until day 63 postoperatively, the end of the autotomy observation period. The drug washout period enabled us to see which of the drugs retained analgesic efficacy after cessation of the treatment. The drugs were compared against two control groups – a group receiving the vehicle and a group not treated at all.

Methods

Surgery: The lumbar spinal cord was exposed by a limited T12, L1 laminectomy. The dura was opened and dorsal roots L3-6 were identified and transected. Extreme care was taken to avoid injury to the spinal cord during the laminectomy and rhizotomy, by transecting the roots as far distally as possible from the spinal cord to not injure the root entry zone. The wounds were then closed in layers. Crystalline Penicillin (5,000 i.u.) was injected i.m. once daily for 3 days postoperatively.

Drug administration: Rats were weighed daily to determine the dose as per body weight. The drugs (at a concentration of 5ml/kg), and vehicle and distilled water were administered twice daily (once between 8:00-9:00 in the morning and once in the evening between 16:00-17:00) *per os* via oral gavage (1 ml).

➢***Preoperative treatment mode:*** For 7 days preoperatively rats received ralfinamide (160mg/kg/d) or pregabalin (20mg/kg/d) or the vehicle or no treatment. On the day of surgery, the last dose for ralfinamide or pregabalin was administered half an hour and 2 hrs before the anesthesia, respectively, so that the occurrence of injury discharge during the operation would be under peak plasma concentration of these compounds. The last gavage with the vehicle was administered half an hour before anaesthesia. No treatment was administered after the surgery to any of these groups, except antibiotics injected to all rats.

➢***Postoperative treatment mode:*** Beginning on the first evening after the surgery, and for 42 days thereafter, rats received ralfinamide (120mg/kg/d), pregabalin (30mg/kg/d), or vehicle; the last group did not receive any treatment. Thereafter, rats continued to be monitored for autotomy during a drug washout period of 21 days, in a study design that we used in previous studies.

➢***Doses:*** These drug doses were selected based on preliminary results (data not shown here) in which the same doses (ralfinamide at 160mg/kg/d and pregabalin at 20mg/kg/d - for the preoperative experiment, and ralfinamide at 120mg/kg/d and pregabalin at 30mg/kg/d – for the postoperative treatment mode) significantly suppressed autotomy following peripheral neurectomy. Moreover, these doses are in the range that shows clinical analgesic efficacy in neuropathic pain.

➢***Autotomy scoring:*** Levels of autotomy were scored daily from day 1 to 63 PO using an acceptable scale (Wall et al., 1979). The animal was gently lifted by the experimenter, the deafferented hindpaw was observed for a few seconds, and autotomy scored by assigning one point for removal of at least 2 nails, and an additional point tallied for every ½ toe injured, to a maximum of 11 (if the animal injured all 5 nails and toes = 5 toes × 2 points/toe +1 point for the nails). Some animals self mutilated the skin or even muscle of the ipsilateral thigh area, in which cases 4 points or 6 points were added for such cases, respectively. The behavioral end point of autotomy was a score of 11. Rats reaching or exceeding this score were euthanized and their score retained with the group's scores for the remainder of the behavioral follow-up until day 63.

Data analysis

Assessment of drug treatment was done by comparing the following parameters of the autotomy behavior:

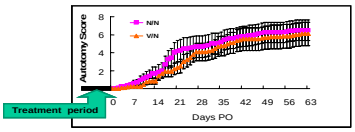
(i) Autotomy onset day (AOD): This parameter was defined as the first day any score of autotomy was detected. Since the paw was totally deafferented, losing one nail could be due to neglect of an anesthetic paw. Therefore, the earliest sign of autotomy was scored when at least two injured nails were detected. Animals expressing no autotomy by day 63 PO (for the Preemptive Study) or day 42 PO (for the Palliative Study) were assigned an arbitrary AOD value of 43 or 64 (respectively). A significantly delayed average AOD in the drug treated groups compared to the control group was considered an expression of analgesia of the tested drug.

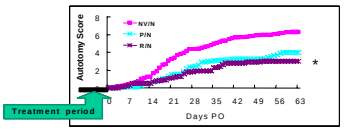
(ii) The course of autotomy (AUC): The average autotomy group scores on d63 (for the Preemptive Study) or d42 and d63 (for the Palliative Study) were compared across groups. Statistically significant lower group average scores compared to the control group indicated analgesia. Integrating the scores of autotomy over the PO days into an 'area-under-the-curve' (AUC) of the autotomy scores over the PO period provided another overall value of the course of autotomy. Statistically significant lower group average AUC values compared to the control group indicated analgesia.

(iii) Incidence of high autotomy (IHA): The % of animals per group that expressed on d63 (for the Preemptive Study) or d42 and d63 (for the Palliative Study) high autotomy scores (≥ 8) was compared across control and treatment groups. Statistically significant lower IHA scores, compared to the control group, indicated analgesia.

(iv) Retaining analgesia after cessation of treatment: The possibility that the treatment suppressed autotomy beyond its administration period was evaluated by calculating the AUC for the period d43–d63, and the incidence of high autotomy on d63. A significantly lower average AUC or a lower IHA, compared to the control group, suggested that drug treatment conferred analgesia that outlasted its administration period.

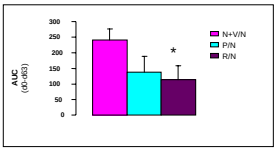
Results of the preemptive analgesia experiment

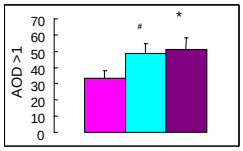


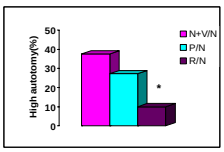


The VHCL (V/N) and no treatment (N/N) groups had identical course of autotomy indicating that daily gavage with the vehicle had no effect on autotomy. Therefore, the two groups were combined into one N+V/N group.

Treatment with both drugs for 7 days before dorsal rhizotomy had a preventive effect on the autotomy behavior which was significant for ralfinamide.







The preemptive effect manifested in significantly reduced course of autotomy (AUC).

The preemptive effect was also expressed by a significant delay in the onset of autotomy for ralfinamide and a trend toward this effect in pregabalin.

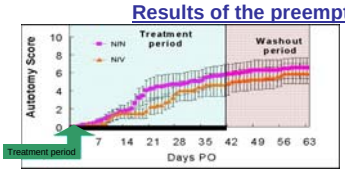
Pretreatment with ralfinamide buyt not pregabalin suppressed the incidence of high autotomy scores on day 63 PO.

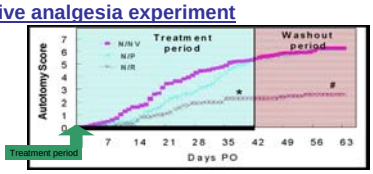
Drugs	Ralfinamide	Pregabalin
Groups	R/N	P/N
AOD (or AOD > 1)	*	#
AUC (d1-d63)	*	
Score d63 PO	*	
High autotomy	*	

Conclusion I

At the tested doses which were selected on the basis of their efficacy to preempt autotomy following peripheral neurectomy, ralfinamide, and less so pregabalin, had a significant *preemptive* analgesic effect in the Dorsal Rhizotomy model as well.

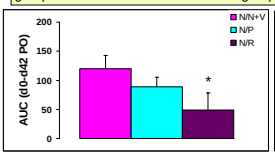
Results of the preemptive analgesia experiment

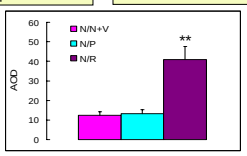


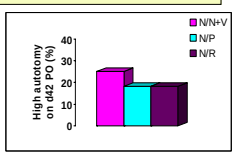


Like the preemptive experiment, the VHCL (V/N) and no treatment (N/N) groups did not differ significantly in any parameter of autotomy indicating that daily gavage with the vehicle for 42 days had no effect on autotomy during the treatment period nor thereafter. Therefore, the two groups were combined into one N/NV group.

Treatment with ralfinamide for 42 days after dorsal rhizotomy had a significant palliative effect on the autotomy behavior.







The postoperative treatment manifested in a significantly reduced course of autotomy (AUC) only in the ralfinamide but not the pregabalin groups.

This effect of ralfinamide was the result of a significant delay in the autotomy onset for the ralfinamide but not the pregabalin treatments.

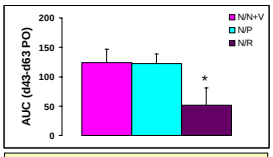
However, these treatments did not affect the incidence of high autotomy scores on day 42 PO.

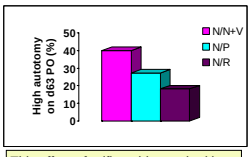
Drugs	Ralfinamide	Pregabalin
Groups	N/R	N/P
AOD	**	
AUC (d0-d42)	*	
Score d42 PO	*	
High autotomy d42 PO		

Conclusion II

At the tested doses which were selected on the basis of their efficacy to suppress autotomy following peripheral neurectomy, ralfinamide, but not pregabalin, had a significant and robust palliative analgesic effect against spontaneous neuropathic pain in this model.

Do the drugs have long lasting analgesic effect ?





Stopping the treatment with ralfinamide retained a low course of autotomy for 3 weeks thereafter. This was not seen in the pregabalin group.

This effect of ralfinamide resulted in a non-significant reduction in the incidence of high autotomy scores.

Drugs	Ralfinamide	Pregabalin
Groups	N/R	N/P
AUC (d43-d63PO)	*	
Score d63 PO	#	
High autotomy d63 PO		

Conclusion III

At the tested doses, ralfinamide, but not pregabalin, retained its significant analgesic efficacy for 3 weeks after stopping the drug treatment.

Summary

Using a model of spontaneous neuropathic pain that is solely driven by abnormal activity in pain pathways in the CNS, we found that:

(1) At the doses tested here, ralfinamide had a slightly better analgesic effect over pregabalin in the preemptive treatment mode and a significantly stronger effect than pregabalin in the palliative treatment mode.

(2) Ralfinamide analgesia operates in part on a target in the CNS.

(3) These results provide a rationale for a clinical trial testing the analgesic effects of ralfinamide in central pain syndromes.

Acknowledgements

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