Axonal protection in experimental autoimmune neuritis by the sodium channel blocking agent flecainide

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Summary
Inflammatory demyelinating neuropathies such as Guillain-Barré syndrome (GBS) and its animal model, experimental autoimmune neuritis (EAN), are typically acute monophasic diseases of the PNS that can leave affected individuals with permanent disability due primarily to axonal degeneration. The mechanisms underlying the degeneration are not understood, but we have previously shown in vitro and in vivo that axons can degenerate when exposed to the inflammatory mediator nitric oxide, and that axons can be protected by application of the sodium channel-blocking agent, flecainide. Here we examine whether flecainide administration can similarly reduce axonal degeneration in the periphery in animals with EAN. EAN was induced in Lewis rats (n = 116, in three independent trials), and rats received either flecainide (Flec) (30 mg/kg/day) or vehicle (Veh) from the onset of disease expression. Flecainide administration significantly reduced the mean (SD) scores for neurological deficit at both the peak of disease (Flec: 5.7 (2.7), Veh: 8.0 (3.6), \( P < 0.001 \)) and at the termination of the trials 25–29 days post-inoculation (Flec: 2.2 (2.4), Veh: 4.2 (4.2), \( P < 0.001 \)). Histological examination of the tibial nerve of EAN animals revealed that flecainide provided significant protection against axonal degeneration so that 80.0% of the normal number of axons survived in flecainide-treated rats compared with 62.8% in vehicle-treated rats (\( P < 0.01 \)). These findings may indicate a novel avenue for axonal protection in GBS and other inflammatory demyelinating neuropathies.

Keywords: EAN; GBS; Guillain-Barré syndrome; neuroinflammation; axonal degeneration

Abbreviations: CAP = compound action potential; CMAP = compound muscle action potential; EAE = experimental autoimmune encephalomyelitis; EAN = experimental autoimmune neuritis; dpi = days post-inoculation; GBS = Guillain-Barré syndrome


Introduction
Guillain-Barré syndrome (GBS) is typically an acute, inflammatory and demyelinating neuropathy that afflicts ~1–4 people per 100 000 worldwide. There are several sub-types of GBS, all of which can involve severe neurological disability and incomplete recovery (Hahn, 1998; Hughes et al., 1999). Axonal degeneration can be pronounced (Feasby et al., 1986; McKhann et al., 1993; Griffin et al., 1996; Rees et al., 1998) and is the main cause of the persistent neurological deficit (Hahn, 1998; Rees et al., 1998).

The causes of axonal degeneration in GBS are poorly understood, but degeneration secondary to severe inflammation has been demonstrated in longitudinal nerve biopsy studies (Feasby et al., 1993). A correlation between the magnitude of inflammation and axonal degeneration has also been reported in experimental autoimmune neuritis (EAN), the animal model of GBS (Hahn et al., 1988). We have previously shown in vitro and in vivo that exposure of axons to the inflammatory molecule, nitric oxide, results in axonal degeneration (Smith et al., 2001; Kapoor et al., 2003), possibly mediated by intra-axonal accumulation of sodium and calcium (Stys et al., 1991, 1992; LoPachin and Lehning, 1997). Axons are particularly vulnerable to degeneration if they are stimulated during exposure to nitric oxide (Smith et al., 2001), as will occur as axons conduct physiological impulse traffic.
through inflammatory lesions. We, and others, have shown that sodium channel blockade can protect axons from nitric oxide mediated degeneration (Garthwaite et al., 2002; Kapoor et al., 2003). Furthermore, the sodium channel blocking agents flecainide (Bechtold et al., 2004) and phenytoin (Lo et al., 2002, 2003) have been shown to be effective in reducing axonal degeneration during experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis.

Here we examine whether flecainide administration is effective in protecting axons from degeneration in EAN. Animals with EAN exhibit many of the pathological features of GBS including multifocal demyelination, inflammatory cell infiltration and axonal degeneration (Hartung and Toyka, 1990; Hahn, 1996; Schmidt et al., 1996). To ensure that the findings would be relevant with regard to potential therapy, we delayed the administration of flecainide until the onset of disease expression. The results show that administration of flecainide to rats with EAN significantly reduced the severity of the neurological deficit, significantly increased the number of functional axons as indicated by electrophysiological examination, and significantly decreased axonal loss. These findings imply a role for sodium channels in axonal degeneration during neuroinflammatory disease, and suggest that flecainide and perhaps agents with similar properties may provide an effective therapy for disorders such as GBS.

Material and methods

Induction of EAN

EAN was induced in female Lewis rats (160–190 g; Harlan, Bicester, Oxfordshire, UK) by a subcutaneous injection at the base of the tail of an emulsion of incomplete Freund’s adjuvant (50 µl; Sigma, Poole, Dorset, UK), bovine peripheral myelin (7.5 mg) and Mycobacterium tuberculosis (1 mg; Difco, West Molesey, Surrey, UK). A high dose of myelin was used to ensure a high degree of axonal degeneration in control EAN rats (Hahn et al., 1988).

Assessment of neurological deficit

Animals were weighed and assessed daily for neurological deficit on a 15-point scale, receiving one point for each of the following signs: 5% weight loss over two days; piloerection; loss of tail tip muscle tone; loss of total tail muscle tone; tail paralysis; decreased toe spread; unsteady gait; one point per hind limb dragged; one point per limb paralysed; moribund; and death. The effect of flecainide on the outcome of EAN was examined in three independent trials (total n = 116; Table 1). The drug administration and daily assessment of neurological deficit were conducted by investigators blinded to the treatment regimen.

Flecainide administration

At the onset of disease expression, rats with similar neurological deficit were paired and assigned to receive either flecainide acetate (Tambocor injection; 3 M, Loughborough, UK; in 2.5% glucose containing 20 mM HEPES, pH 7.4, at 30 mg/kg/day) or vehicle.

| Table 1 Deficit protection, electrophysiology and axon counts for EAN rats |
|-------------------------|-----------------|----------------|-------------|
|                         | Vehicle         | Flecainide     | P           |
| Peak deficit score      |                 |                |             |
| Trial 1 18 8.2 (5.9)    | 17 6.1 (3.7)    | 0.166         |
| Trial 2 19 9.4 (3.3)    | 20 6.0 (2.8)    | 0.001 <0.001  |
| Trial 3 21 6.6 (1.9)    | 21 5.1 (1.6)    | 0.010         |
| Mean 58 8.0 (3.6)       | 58 5.7 (2.7)    | 0.007         |
| Cumulative deficit score|                 |                |             |
| Trial 1 18 77.7 (56.9)  | 17 48.9 (35.4)  | 0.064         |
| Trial 2 19 83.0 (29.2)  | 20 49.5 (25.5)  | 0.001 <0.001  |
| Trial 3 21 56.3 (23.7)  | 21 37.7 (18.6)  | 0.007         |
| Mean 58 71.7 (39.7)     | 58 45.1 (26.9)  | 0.007         |
| Terminal deficit score  |                 |                |             |
| Trial 1 18 5.5 (5.5)    | 17 3.4 (3.3)    | 0.186         |
| Trial 2 19 5.3 (3.7)    | 20 2.6 (1.6)    | 0.006 <0.001  |
| Trial 3 21 2.2 (2.2)    | 21 0.8 (1.1)    | 0.011         |
| Mean 58 4.2 (4.2)       | 58 2.2 (2.4)    | 0.007         |
| CAP area (µV·msec)      |                 |                |             |
| Trial 1 8a 8.7 (17.2)   | 10b 22.6 (40.1) | 0.380         |
| Trial 2 15 32.2 (30.8)  | 18 98.4 (60.2)  | <0.001 <0.001 |
| Trial 3 21 55.7 (42.5)  | 21 77.4 (47.5)  | 0.126         |
| Mean 44 39.1 (33.9)     | 49 73.9 (50.6)  | 0.007         |
| CMAP area (mV·msec)     |                 |                |             |
| Trial 1 8a 17.0 (9.8)   | 10b 19.9 (10.1) | 0.555         |
| Trial 2 17 8.3 (6.6)    | 20 15.2 (6.8)   | 0.004 0.001   |
| Trial 3 21 15.3 (9.4)   | 21 19.5 (8.0)   | 0.130         |
| Mean 46 13.0 (8.5)      | 51 17.9 (7.9)   | 0.007         |
| Axons (% of normal)     |                 |                |             |
| Trial 1 10a 54.3 (34.6) | 10b 70.0 (20.7) | 0.236         |
| Trial 2 10b 50.1 (23.9) | 10b 81.0 (16.3) | 0.004 <0.001  |
| Trial 3 19 73.9 (19.1)  | 18 85.2 (20.1)  | 0.050         |
| Mean 39 62.8 (26.7)     | 38 80.0 (19.9)  | 0.007         |

Peak deficit score is the maximum deficit score received at any time during the trial (1–29 dpi) with lethal EAE as 15. Cumulative deficit score is the sum of the daily deficit scores with lethal EAE as 15. Terminal deficit score is the deficit score received on the final day of the trial with lethal EAE recorded as 15. The eight most severe EAN rats used for analysis. The ten most severe EAN rats used for analysis.

Dosing was by subcutaneous injection twice a day until the end of the experiment, 25–29 days post-inoculation (dpi). All the experiments were approved by the local ethics committee King’s College London and were licensed under the Animals (Scientific Procedures) Act 1986 of the UK Home Office.

Electrophysiological examination

At the termination of the trials (25–29 dpi), the animals were anaesthetized (2% halothane in oxygen) and examined electrophysiologically. The skin of the back was incised and a stimulating cathode was inserted in the paravertebral muscle at the T9/T10 vertebral junction, with the anode positioned over the right shoulder blade. ‘Active’ and ‘indifferent’ recording electrodes were positioned percutaneously at the base of the tail and the tail tip, respectively. A ground electrode was inserted subcutaneously over the caudal end of the pelvis. Electrical stimuli (80 V, 20 µsec) were applied at 1 Hz and averaged (n = 16) compound potentials were recorded digitally. After recording the directly conducted sensory axonal compound action potential...
(CAP) evoked by stimulation of the dorsal columns, the stimulating cathode was moved to the paravertebral muscle at the L6/S1 vertebral junction so that the compound muscle action potentials (CMAPs) could be recorded. The records were analysed quantitatively using our own purpose-written software.

**Histological examination**

**Trials 1 and 2**

Following the electrophysiological recording, the tissues were fixed for histological examination by transcardiac perfusion with rinse (0.9% saline containing 10 mM HEPES, 0.05% lignocaine, 2 U/ml heparin, 0.02% NaNO₂), followed by 3.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4). Sciatic nerves were removed and stored in the same fixative at 4°C. Transverse 0.5 mm blocks were cut from the same location along the sciatic nerve in each animal and embedded into TAAB resin (TAAB Laboratories Equipment Ltd, Aldermaston, Berkshire, UK) as described previously (Redford et al., 1995).

**Trial 3**

Tibial nerves were collected fresh and fixed by immersion in 3.5% glutaraldehyde in 0.15 M phosphate buffer. Transverse 0.5 mm sections were collected and embedded as above. Following resin embedding, semi-thin sections (0.8 μm) were cut, collected onto slides, and stained with toluidine blue and pyronin Y. Assessment of axonal loss in EAN rats was carried out on the tibial nerve. Myelinated and demyelinated axons were marked and counted on high magnification digital photographs of nerve sections using SigmaScan digital analysis software (SYSTAT Software Inc, Richmond, California, USA). Following trials 1 and 2, axonal diameter was measured in naïve (n = 4) and severely affected EAN rats (n = 40) to compare the magnitude of axonal degeneration in axons of different diameter. In order to assess the magnitude of endoneurial inflammation in the EAN animals, macrophages were marked and counted on nerve sections stained with toluidine blue and pyronin Y, which had been previously used for axon counting in trials 1 and 2.

**Detection of anti-myelin antibodies**

The presence of anti-peripheral myelin antibodies was determined by enzyme-linked immunosorbant assay (ELISA) using a method adapted from Challacombe et al. (1986). Briefly, bovine peripheral myelin (50 μg/ml containing methylglyoxal (0.3%, Sigma) was incubated in 96-well ELISA plates (Linbro EIAII supplied by Flow Laboratories) at 37°C for 2 h. Plates were washed carefully and non-specific binding blocked with fish gelatin (1%, Sigma) in phosphate buffered saline (PBS pH 7.4). Rat serum (50 μl/well) was added at 1 to 50, 100, 200, 400 and 800 dilutions in PBS and incubated overnight at 4°C. Myelin reactive antibodies were detected using alkaline phosphatase-conjugated goat anti-rat IgG (1 : 1000, Sigma), followed by p-nitrophenyl phosphate. Plates were read at 405 nm on an Anthos plate reader (Jensons PLS, East Sussex, UK). Samples were measured in triplicate and the anti-myelin antibody titre expressed as the serum dilution at which the sample absorbance was twice that of the background.

**Statistics**

The significance of the differences between the responses in drug treated and controls in our outcome measures (deficit score, electrophysiology and axon counts) in each individual trial was calculated using a Mann–Whitney U-test (Table 1). To examine the combined significance of the three trials, a pooled analysis using the weighted mean difference was performed using a Student t-test.

For graphical illustration and comparison with normal Lewis rats, data from the three trials were combined and presented as a cumulative mean (with SDs) and tested for significance using ANOVA (analysis of variance). When multiple comparisons were made using ANOVA, a post hoc Dunn’s test was used. Spearman’s rank correlation test was used to examine correlations.

**Results**

**Neurological disability**

Flecainide administration significantly reduced the severity of the neurological deficit resulting from EAN when compared with rats treated with vehicle (Fig. 1A). For statistical purposes, the significance of this effect was assessed using the
scores of individual rats to obtain mean scores for peak, cumulative and terminal deficit (Fig. 1B). The results from the three trials are reported individually and as a weighted mean difference in Table 1.

Over the three trials, rats with EAN exhibited a mean (±SD) peak deficit score of 8.0 ± 3.6 (equivalent to tail paralysis and hind limb weakness); treatment with flecainide significantly reduced this deficit to a score of 5.7 ± 2.7 (reduced muscle tone in the tail, normal hind limb function) (P < 0.001). In addition, vehicle-treated rats with EAN exhibited an incomplete recovery of function by the end of the trials as shown by a mean terminal deficit score of 4.2 ± 4.2 (equivalent to persistent weakness of the tail, Fig. 1B), whereas animals treated with flecainide had very little persistent deficit (terminal deficit score of 2.2 ± 2.4, P < 0.001).

Electrophysiological examination

Compared with normal rats (164.5 ± 41.4 µV·msec, n = 6), the area of the CAP evoked by stimulation of the dorsal columns (Fig. 2A) was reduced in vehicle-treated rats to only 39.1 ± 33.9 µV·msec (n = 44; P < 0.001). However, the area of the CAP was significantly higher (73.9 ± 57.5 µV·msec; n = 49; P < 0.05; Fig. 2C) in animals with EAN treated with flecainide. A similar beneficial effect of flecainide was observed when measuring the CMAP resulting from stimulation of the lumbar spinal roots (Fig. 2B, D). Whereas the CMAP area was 13.0 ± 9.1 mV·msec in vehicle treated animals with EAN, it was significantly greater at 17.9 ± 8.1 mV·msec in animals treated with flecainide (P < 0.05). Sensory conduction (CAP area evoked by dorsal column stimulation) in rats with EAN was reduced to a greater extent (76% reduction from normal) than motor conduction (CMAP area), which

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**Fig. 2** Electrophysiological examination of axonal conduction in rats with EAN 25–29 dpi. (A, B) Representative records from normal Lewis rats and rats with EAN treated with vehicle or flecainide following thoracic (A) and lumbar (B) stimulation, and resulting in sensory axon and muscle CAPs, respectively. The region used to calculate the area of the records is demonstrated (thick dashed line) on the normal CAP and CMAP traces. (C, D) The mean area of the CAP (C) and CMAP (D) recorded from rats with EAN which were treated with vehicle was significantly reduced from normal. However, flecainide treatment resulted in significantly greater CAP and CMAP areas. Dashed line in C and D = mean CAP/CMAP area recorded in normal Lewis rats. Bars = SD. *P < 0.05; **P < 0.01.
was reduced by 41%. Flecainide was also more effective at preserving sensory conduction during EAN, such that CAP area was improved by 87% in flecainide-treated rats (relative to vehicle-treated ones), while CMAP area increased by only 36%. Previous work in our laboratory has shown that flecainide administration in normal rats does not noticeably affect the area of CAP or CMAP (Bechtold et al., 2004).

**Axonal protection**

Myelinated and demyelinated axons were easily identified on toluidine blue-stained, transverse sections through the tibial nerve (Fig. 3), allowing the number of surviving axons to be counted directly. Vehicle-treated rats with EAN showed a pronounced loss of axons and evidence of ongoing axonal degeneration (Fig. 3C–F); in severe cases, almost all the

![Fig. 3 Tibial nerve pathology. Typical tibial nerve sections stained with toluidine blue and pyronin Y collected from naïve Lewis rats (A, B), and rats with EAN treated with either vehicle (C–F) or flecainide (G, H). Rats with EAN treated with vehicle exhibited evidence of axonal degeneration (C, D), which resulted in almost complete loss of tibial nerve axons in rats with severe disease pathology (E, F). This loss was greatly reduced in rats with EAN treated with flecainide, although some evidence of axonal degeneration was still observed (G, H). Bar = 80 μm in A, C, E and G; 15 μm in B, D, F and H.](image-url)
tibial nerve axons had degenerated (Fig. 3E, F). Axonal pathology was greatly reduced in animals treated with flecainide (Fig. 3G, H). Quantification of axonal survival revealed that, whereas on average almost 40% of axons degenerated in vehicle-treated control rats with EAN (remaining axons were 61.8 ± 26.7% of normal; Flec: 72.5 ± 21.5%; Veh: 60.2 ± 38.7%; P = 0.22), flecainide treatment protected almost half of them (80.0 ± 19.9% of normal axon numbers).

The axonal protection conferred by flecainide treatment was most noticeable when the small (<4 μm) and large (>4 μm) diameter axons were considered separately (Fig. 4B). Small diameter axons were disproportionately affected such that almost 60% of them underwent degeneration in vehicle-treated rats (surviving axons were 44.5 ± 25.3 of normal), but most of these axons were protected by flecainide treatment (77.0% ± 29.2 of normal; P < 0.01). In contrast, while flecainide did increase the number of surviving large diameter axons following EAN, the level of protection did not reach statistical significance (Flec: 72.5 ± 21.5%; Veh: 60.2 ± 38.7%; P = 0.22).

The number of surviving axons showed a strong negative correlation (r = −0.76, P < 0.01) with the cumulative score of neurological deficit exhibited by rats with EAN. (D) The mean number of demyelinated axons in the tibial nerve of rats with EAN was low and no significant difference was detected between the groups. Bars = SD.
Reduction in myelin reactive antibodies

As a possible measure of the magnitude of the immune response in the rats with EAN, serum isolated from naïve and diseased rats was tested for antibodies reactive to peripheral myelin. As illustrated in Fig. 5, the mean (±SD) titre of serum anti-myelin antibodies in rats with EAN treated with flecainide (titre (1/x): 647.4 ± 258.5) was significantly lower than that found in EAN rats treated with vehicle (1038.5 ± 420.9, P < 0.01).

Reduction in macrophage infiltration

Macrophages were marked and counted on tibial nerve sections collected from severely affected rats with EAN from trials 1 and 2 (n = 37). Significantly fewer macrophages were observed in the tibial nerves of rats with EAN treated with flecainide compared with rats with EAN and treated only with vehicle (Flec: 112.6 ± 86.9 cells/section; Veh: 233.4 ± 169.8; P < 0.01) (Fig. 6A). Macrophage infiltration demonstrated a significant correlation with the extent of axonal degeneration observed in the tibial nerves (r = −0.87, P < 0.01). Bars = SD. **P < 0.01.

Discussion

The findings show that flecainide treatment provides a significant level of protection in EAN with regard to the severity of the neurological deficit and the loss of axons, as assessed by measurement of the area of compound action potentials and by counting the number of intact axons in the tibial nerve. This beneficial effect of flecainide was shown consistently over three independent trials. EAN is believed to be a good model for GBS and, as axonal degeneration is the major cause of the slow and incomplete recovery of disability in GBS (Feasby et al., 1993; Rees et al., 1998), the current findings suggest that flecainide may protect axons in patients with GBS.

Flecainide is a well-characterized sodium channel blocking agent (Mueller and Baur, 1986; Ragsdale et al., 1996; Liu et al., 2002), and we have hypothesized that its protection of axons during EAN is mediated, in part at least, by a direct action on axonal voltage-gated sodium channels. We propose that the partial blockade of sodium currents by flecainide averts a deleterious axoplasmic accumulation of sodium at sites of inflammation, which in turn prevents a damaging rise in intra-axonal calcium ions due to reverse operation of the Na⁺/Ca²⁺-exchanger (Stys et al., 1991, 1992; Fern et al., 1993; reviewed in Bechtold and Smith, 2004). This mechanism is supported by several lines of research that implicate a role for the intracellular accumulation of sodium ions in axonal degeneration (LoPachin and Lehning, 1997). Several pathological features of EAN, such as heightened nitric oxide production and demyelination, would be expected to increase sodium loading in PNS axons.

Sodium loading may be anticipated at sites of inflammation due to the prominent expression of the inducible form of nitric...
Axonal sodium loading could be further enhanced by demyelination. For example, demyelinated axons acquire an increased expression of sodium channels along the demyelinated axolemma, associated with a several fold increase in channel density within lesions (Foster et al., 1980; England et al., 1990, 1991; Novakovic et al., 1998). Although this development can be beneficial in terms of the restoration of conduction to the axon (Bostock and Sears, 1976; Smith et al., 1982), it may also dramatically increase the sodium load experienced by axons when conducting impulses. Furthermore, demyelinated axons, especially sensory axons, can become hyperexcitable so that they generate persistent trains of impulses at frequencies of almost 50 Hz; the impulses arise ectopically at the site of demyelination (reviewed by Smith and McDonald, 1999). Such activity can be expected to dramatically increase sodium entry into axons, and thus sodium channel blocking agents may be especially beneficial in protecting demyelinated axons.

A preferential degeneration of small diameter fibres has been reported in EAN (Duckers et al., 1994); these axons were also disproportionately affected in our study. Small axons are also severely affected in white matter tracts of patients with multiple sclerosis (Ganter et al., 1999; Evangelou et al., 2001). It is interesting that the protective effect of flecainide is particularly prominent in small diameter axons (<4 μm), such that the number of surviving small diameter axons in flecainide-treated rats is increased by an average of 73.0% (relative to rats treated with vehicle) compared with an increase in the average number of large diameter axons of only 20.4%. It is not clear why small axons are particularly vulnerable to degeneration in EAN or particularly protected by flecainide. However, small diameter axons have an increased vulnerability to anoxia (Lehning et al., 1995) and therefore may be particularly sensitive to sodium-mediated degeneration.

These findings were paralleled by the electrophysiological data, which revealed a disproportionate loss of conduction in sensory axons during EAN and a disproportionate preservation of function in sensory axons with flecainide administration. Small diameter axons are typically sensory in function and sensory axons are known to have an increased expression of persistent sodium currents (Bostock and Rothwell, 1997); this current also predisposes them to hyperexcitability (Bostock and Rothwell, 1997; Kapoor et al., 1997; Segal and Douglas, 1997). The particularly marked effect of flecainide for small axons may be related to the fact that flecainide is potent in blocking the persistent sodium current, at least in cardiac (Na\textsubscript{v}1.5) and smooth muscle (Na\textsubscript{v}1.4) sodium channels (Nagatomo et al., 2000; Wang et al., 2003).

Flecainide not only increased the number of surviving axons, but also the number of functioning axons (as assessed electrophysiologically) in rats with EAN. The area of both the CAP and CMAP was significantly greater in the animals treated with flecainide. This finding is in agreement with the observation that these animals exhibited a reduced neurological deficit compared with vehicle treated. There is a concern that the use of sodium channel blocking agents in patients with inflammatory demyelinating disease may contribute to a worsening of neurological deficits by promoting conduction failure in axons with reduced safety factor (Sakurai et al., 1992; Sakurai and Kanazawa, 1999). It is therefore notable that the current findings indicate that any such impairment may be than compensated for by the increase in axonal survival.

Demyelinated axons were present in the tibial nerves of rats with EAN, and no significant difference in their number was observed between the vehicle and flecainide-treated groups, suggesting that flecainide does not affect this component of the pathology. However, it is possible that flecainide may affect Schwann cell function. Indeed, voltage-gated sodium channel expression has been demonstrated in rabbit (Chiu et al., 1984; Shrager et al., 1985; Belcher et al., 1995), rat (Konishi, 1990; Rothstein et al., 1994) and human (Kamleiter et al., 1998) Schwann cells in vitro. However, sodium channel expression appears to be limited to non-myelinating Schwann cells (Chiu, 1987; Wilson and Chiu, 1990; Toledo-Aral et al., 1997), suggesting that flecainide may have little effect on the myelinating Schwann cells which may be affected immunologically in this study.

The finding that significantly fewer macrophages were present in the nerves of flecainide-treated rats is interesting, but the cause is uncertain. Only one time point was sampled (the end of the trial) and so it is not certain whether flecainide reduced macrophage recruitment per se or simply reduced the stimulus for macrophage recruitment by reducing axonal degeneration. Flecainide may have a previously unrecognized ability to suppress immune or inflammatory responses. If true, flecainide may protect axons by acting as an anti-inflammatory...
agent, possibly altering T cell, B cell or macrophage activation. A role for sodium currents in T-cell activation and co-stimulation has been suggested (DeCoursey et al., 1985; Khan and Poisson, 1999; Lai et al., 2000) and fast transient sodium currents have been observed in several human T cell lines (Gallin, 1991). With regard to B cells, the current findings revealed a reduction in the titre of anti- peripheral myelin antibodies in the sera of rats with EAN treated with flecainide. Again, this might have been due to the reduction of exposure to myelin breakdown products as a consequence of the lesser degree of axonal degeneration. However, a direct effect of the drug on antibody production cannot be excluded. Amiloride and tetrodotoxin sensitive sodium channels have been reported on human and rat B cells (Pieri et al., 1989; Bubien and Warnock, 1993; Bradford et al., 1995; Oh and Warnock, 1997) and sodium channel blockade with amiloride inhibited antibody secretion by lymphocyte hybridomas (Zhou and Bubien, 2002). As anti- peripheral myelin antibodies have been shown to contribute to demyelination and clinical disability in EAN (Taylor and Pollard, 2001, 2003), the reduction in the titre of these antibodies with flecainide treatment may contribute to the improved outcome observed in treated rats in our study. Sodium currents have also been implicated in macrophage activation and effector functions. Amiloride-sensitive sodium channels have been identified on rat peritoneal macrophages (Negulyaev and Vedernikova, 1994) and amiloride administration suppresses cytokine release from human alveolar macrophages (Rolfe et al., 1992). In addition, the sodium channel blocking agent lignocaine has been shown to inhibit superoxide release from rabbit alveolar macrophages (Bidani and Heming, 1997).

In conclusion, the present report demonstrates that the sodium channel blocking agent, flecainide, can significantly protect axons from degeneration in a model of GBS. It therefore follows that agents such as flecainide may represent a novel therapeutic avenue for the treatment of GBS and related diseases.

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References


Hahn AF. Experimental allergic neuritis (EAN) as a model for the immune-mediated demyelinating neuropathies. Rev Neurol (Paris) 1996; 152: 328–32.


Nagatomo T, January CT, Makielski JC. Preferential block of late sodium current in the LQT3 Deltakpoq mutant by the class I(C) antiarrhythmic flecainide. Mol Pharmacol 2000; 57: 101–7.


Segal MM, Douglas AF. Late sodium channel openings underlying epileptiform activity are preferentially diminished by the anticonvulsant phenytoin. J Neurophysiol 1997; 77: 3021–34.


