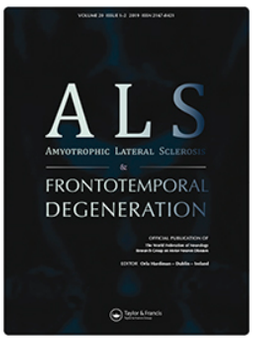

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RESEARCH ARTICLE

ALSUntangled 46: penicillin G/hydrocortisone

THE ALSUNTANGLED GROUP

ALSUntangled reviews alternative therapies on behalf of persons with ALS (PALS). Here we review the combination of penicillin G and hydrocortisone (PNG/HC) as a treatment for ALS, for which we have had over 600 requests (1).

Overview

Penicillin G (benzylpenicillin; PNG) is a natural member of the β-lactam class of antibiotics which today is most often used against syphilis and susceptible *Streptococcus* infections (2). PNG has been reported to decrease the effect of the inhibitory neurotransmitter GABA (ɤ-aminobutyric acid) *in vitro* (3) through inhibition of GABA receptors (4–7), inhibition of GABA molecular synthesis (8), and inhibition of GABA synaptic release (9,10). PNG may also decrease excitotoxicity caused by the neurotransmitter glutamate as described below.

Hydrocortisone (HC; cortisol) is the main glucocorticoid steroid that humans produce naturally in the adrenal glands. Glucocorticoids have many actions, most of which are either metabolic or immunologic. Glucocorticoids are anti-inflammatory and immunosuppressive. Synthetic glucocorticoids and HC are used in medicine for many endocrine, autoimmune, and inflammatory disorders (11). HC can also affect GABA neurotransmission *in vitro* and in healthy humans (12–14).

In a case series published in 1990, PNG and HC were used to treat five patients with blood tests positive for syphilis and a motor neuron disease resembling ALS (15). Four of these patients subsequently had improved strength and/or function that was maintained through an approximately 5-year follow-up. The fifth had stability of their ALS-like illness through 10-years of follow-up. Since this is not typical of the natural history of ALS, the authors concluded that neurosyphilis could manifest as a potentially treatable ALS-mimic syndrome. Recently, Dr. Bert Tuk, a pharmacology researcher, hypothesized an alternate explanation for the treatment-associated benefits seen in these five patients: that the combination of PNG actions on GABA and HC actions on GABA and neuroinflammation were responsible, while the syphilis infection was only coincidental (16–18). Tuk has applied for a patent to use GABA inhibitors in ALS which includes the PNG/HC regimen (19); he is the founder of Ry Pharma, a company developing GABA inhibitor pharmaceuticals with plans for a future clinical trial (20). We review Dr. Tuk's hypotheses in the "Mechanisms" section. Tuk has also published a case series of three PALS without syphilis who were treated with combination intravenous PNG and HC (21), which we will discuss in the "Cases" section.

Mechanisms

Neuroinflammation

The more studied of the two ALS mechanisms that Tuk proposes PNG/HC to act on is neuroinflammation. Although neuroinflammation is present in ALS, its role remains controversial. There may be components of the inflammatory response that are protective against ALS while other parts may drive ALS disease progression (22). Even if a component of neuroinflammation is a key driver of the disease, glucocorticoids do not appear to sufficiently modulate it, as previous clinical trials of immunosuppressive regimens that included glucocorticoids failed to show any benefit (23,24).

ALSUntangled Reviewers who contributed to this paper include the following: Jesse Crayle (lead author), Richard Bedlack (senior author), Carmel Armon, Paul Barkhaus, Michael Bereman, Mark Bromberg, Benjamin Rix Brooks, Greg Carter, Amy Chen, Merit Cudkowicz, Jonathan D. Glass, Daniel Harrison, Christopher J McDermott, Kathy Mitchell, Craig Oster, Sabrina Paganoni, Meraida Polak, Colin Quinn, Victoria Rose, Jeffery D. Rothstein, Kristiana Salmon, Fernando Vieira, and Dane Ward. Note: this paper represents a consensus of those weighing in. The opinions expressed in this paper are not necessarily shared by every investigator in this group.
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Excess inhibitory GABA neurotransmission

The second mechanism that Tuk proposes PNG/HC to act on is GABA neurotransmission. Tuk makes the novel proposal that ALS is primarily caused by an increase in inhibitory GABA activity. This results in a compensatory increase of the excitatory neurotransmitter glutamate as a homeostatic response to maintain balance (16–18). In this scenario, PNG/HC would decrease GABA activity, and in turn decrease the homeostatic increase in glutamate excitotoxicity. The idea that glutamate excitotoxicity may play a role in ALS pathophysiology is not new (25), but Tuk's proposed role of GABA as a key driver of disease progression is. In support of his hypothesis, Dr. Tuk makes the following points:

a. Despite elevated CNS levels of the excitatory neurotransmitter glutamate, PALS do not have seizures and therefore there must be increased levels of an inhibitory neurotransmitter such as GABA.
b. PALS do not have "increased muscle activation". Instead they have "complete muscle inhibition in the end stages". Therefore, there must be substantial inhibitory GABA neurotransmission.
c. Pharmaceutical drugs that "increase GABAergic activity" (e.g. benzodiazepines) can produce symptoms similar to ALS such as dysarthria and dysphagia.
d. Well-conducted clinical trials of PALS evaluating "GABAergic compounds" (Tuk cites gabapentin, topiramate, and minocycline as examples) showed faster "disease progression" in the treatment groups compared to placebo.
e. Typical patterns of weakness in PALS, including the split hand phenomenon, are explained by differences in the degree of GABA-mediated inhibition between muscles.

There are problems with Tuk's hypothesis and supporting arguments. The biggest problem is that most studies do not show elevated GABA in the CNS of ALS animal models or in PALS. In fact, studies with the SOD1 G93A mutant mouse model suggest there is decreased GABA in the motor cortex, brainstem, and striatum relative to control mice, that further decreases with disease progression (26). The wobbler mouse model of ALS also shows some signs of decreased cortical inhibitory neurotransmission (27). In PALS, magnetic resonance spectroscopy scans suggest that motor cortex GABA levels are lower than in healthy controls (28). This decrease in GABA appears to be due to the death of GABAergic inhibitory interneurons as PALS at autopsy have less inhibitory interneurons in the motor cortex and other areas when compared to patients that did not have motor neuron disease (29). Regarding Tuk's supporting arguments:

a. Case reports *do* suggest an association of epileptic seizures with ALS, especially in PALS with certain ALS-causing genetic mutations (30–33). There is also clearly an increased risk of seizures in frontotemporal dementia (34), which is pathologically and clinically associated with ALS.
b. Transcranial magnetic stimulation (TMS) studies show that PALS have evidence of cortical hyper- (not hypo-) excitability which precedes the onset of weakness (35,36). TMS also shows that cortical inhibition is decreased compared to healthy controls and decreases further as the disease progresses (36). Electrodiagnostic studies (electromyography; EMG) show that muscles become weak in ALS because of a loss of lower motor neurons and not because of active inhibition (36).
c. Dysarthria and dysphagia are not specific to overuse/overdose of a GABA agonist, e.g. benzodiazepines. There are many possible pathologies that induce these symptoms including stimulant toxicity (37), genetic muscular dystrophies, and autoimmune neuromuscular diseases (38). In ALS, these symptoms are caused by the death of motor neurons.
d. Review of all the data on gabapentin in PALS shows that it is no different than placebo (39). PALS taking topiramate in a clinical trial had faster loss of arm strength than those on placebo; however, there was no difference in ALSFRS (ALS functional rating scale), FVC (forced vital capacity), and survival. The most likely explanation for faster progression on a single outcome measure was the substantial number of adverse events related to topiramate including weight loss, loss of appetite, and psychological depression (40). PALS taking minocycline in a clinical trial had faster progression than those on placebo in several outcome measures. The reason for this is not clear and it is not related to dosage or adverse events (41). Minocycline in cell culture of rat neurons has been reported to decrease glutamate-mediated excitability but does *not* affect GABA neurotransmission (42). Therefore, we believe it is unlikely that that the minocycline result is relevant to this discussion.
e. Very recent work suggests that the split hand syndrome in ALS is not explained by motor neuron excitability, but instead by the reinnervation capacity of different muscle groups (43).

Glutamate excitotoxicity

As mentioned above, excessive glutamate neuroexcitation is thought to be a key pathophysiological feature of ALS (25). PNG and another β lactam (ceftriaxone) have been reported to increase glutamate uptake transporters (EAAT2; GLT-1) in the

Table 1. Table of evidence.

	Grade	Explanation
Mechanism	D	Glucocorticoids have failed to affect neuroinflammation in ways that beneficially alter ALS disease progression in clinical trials. There is little scientific evidence that GABA is contributing to ALS pathogenesis. PNG can increase glutamate uptake and slow ALS progression in an animal model, but a drug that does this more potently did not work in a human ALS trial.
Preclinical	U	We found no studies testing PNG and/or HC in preclinical models of ALS.
Cases	D	A case series of 3 Dutch PALS reportedly experiencing benefits from PNG/HC was published; however, we did not receive medical records to confirm the ALS diagnoses or improvements.
Trials	U	We found no trials testing PNG and/or HC in PALS.
Risks	D	Very rarely, life-threatening side-effects may occur with PNG. In the case series of 3 PALS given PNG/HC infusions, 2 experienced high blood pressure with an associated headache that required treatment.

spinal cord of healthy rats, with an associated decrease in free glutamate. In the SOD1 mutant mouse model of ALS, ceftriaxone increases EAAT2 levels in the spinal cord and slows motor neuron loss and clinical disease progression (44); however, a large phase III clinical trial with ceftriaxone showed no benefit in PALS (45). PNG is moderately less potent than ceftriaxone at increasing EAAT2 levels *in vivo* (44), suggesting that PNG is unlikely to benefit PALS through this mechanism.

In conclusion, glucocorticoids have failed to affect neuroinflammation in ways that beneficially alter ALS disease progression in clinical trials. There is little scientific evidence that GABA is contributing to ALS pathogenesis. PNG can increase glutamate uptake and slow ALS progression in an animal model, but a drug that does this more potently did not work in a human ALS trial. Based on the above discussion, ALSUntangled assigns PNG/HC a "mechanism" grade of D (Table 1).

Preclinical models

We could not find any studies with PNG and/or HC in preclinical models recognized as relevant to ALS. Based on the lack of research, ALSUntangled assigns a "preclinical models" grade of U for PNG/HC (Table 1).

Of potential interest, Tuk cites one study in an ALS preclinical model utilizing glucocorticoids (21). In this study, mutant SOD1 mice were given either saline, the synthetic glucocorticoid steroid methylprednisolone in free form or methylprednisolone in a special molecular packaging. There was no difference in motor performance between treatment groups; however, there were some improvements on MR (magnetic resonance imaging scans) and histology with the specially packaged methylprednisolone (46). This study has not been replicated. The results suggest that free glucocorticoid may have no effect, even if glucocorticoid packaged in a novel delivery system might have some benefit.

Cases

The only published case reports we found describing PALS without syphilis taking PNG/HC were the case series authored by Dr. Tuk (21). He describes three patients reportedly diagnosed with ALS at the Netherlands National ALS Center that were given at least two 21-day rounds of intravenous PNG with a 10-week break between infusions. The PNG was initially titrated up to the target daily dose of 20 million units starting on day five of each treatment round. During the first two weeks of each treatment round, the patients were also infused with 100 mg HC daily. HC was discontinued after day 14 without a taper. Over the course of the first infusion round, Patient 1 reportedly experienced rapid objective improvements in dysarthria, dysphagia, and limb strength. Previously wheelchair bound, he regained the ability to walk 650 m. The patient's improvements were transient and faded over the 10 weeks following the first round of infusion rendering the patient again wheelchair-bound. A second round of infusions again improved his function a small amount; however, he was still unable to walk. In 4 weeks of follow-up after the second round of infusions, he reportedly retained improvements in finger movement, speech, and swallowing. Patients 2 and 3 reportedly experienced similar improvements in speech and swallowing (patient 3 also had improvements in limb strength) over the course of the first infusion which remained unchanged over the next 10-week break, second round of infusions, and few weeks of additional follow-up.

There are some problems with this case series. First, there are not enough details provided to convey confidence about the ALS diagnoses, especially given the described atypical features of dyscoordination and generalized muscle pain in patient 1, tremor and dyscoordination in patient 2, and dyscoordination and a resting tremor in patient 3. We reached out by phone to Dr. Tuk who stated he would attempt to obtain the patients' medical records to send us (47), but we have not yet received them. Second, there are few objectives or quantitative measurements in the reported improvements; there are no reported FVC (forced vital

capacity) respiratory measurements, functional rating scale scores, or muscle strength ratings. In our phone conversation, Dr. Tuk confirmed that none of these measures were obtained. Third, the reported clinical follow-up of these patients is short. Dr. Tuk told us in our phone call that at least two of these cases received additional PNG/HC treatment cycles and months of follow-up, but that the initial improvements in motor function eventually disappeared and the patients did not improve with additional PNG/HC infusion cycles. Finally, there are potential conflicts of interest in regard to this study as its authors have affiliations with Ry Pharma (20).

In our phone call (47), Dr. Tuk stated that there have been an additional 20 PALS whom have received the same treatment he described in his paper. He said that 30% of these cases showed some transient clinical improvement which was most noticeable after the first cycle and became less noticeable and eventually nonexistent after subsequent cycles. A Google search revealed no additional cases of PALS taking PNG/HC and we found no one on PatientsLikeMe reporting the use of this combination or PNG alone for their ALS (48–51). Based on the above discussion, ALSUntangled assigns a TOE "Cases" grade of D (Table 1).

Trials

We found no published trials of PNG/HC in PALS. We did learn of a 16-patient placebo-controlled trial that is underway in Europe (52,53). Based on the lack of currently available data from trials with PNG/HC, ALSUntangled assigns a "trials" grade of U.

Risks

The most common side effects with PNG are mild gastrointestinal distress typically manifesting as diarrhea as well as itching. Side effects may occur up to 10% of the time, but severe effects such as anaphylaxis or antibiotic-associated colitis requiring hospitalization occur in less than 1% of patients (54–56). Glucocorticoids have many associated dose-dependent side-effects from different organ systems including hypertension and adrenal insufficiency (57). In Dr. Tuk's case series, two of the three treated PALS experienced hypertension with associated headaches that required an anti-hypertensive medication to treat and resolved on discontinuation of the HC (21). Based on the available evidence, ALSUntangled assigns PNG/HC a "risks" grade of D (Table 1).

Dosing and costs

We estimate that the treatment costs for two rounds of PNG/HC infusions as described in Dr. Tuk's series would be approximately $6000 for the medication (58,59) and additional facility and staffing costs for administering the infusions.

Conclusion

In our opinion, there is no convincing evidence that GABA overload plays a role in ALS progression. PNG/HC has other theoretical mechanisms by which it could slow ALS progression, but previous human trials involving steroids and a trial of a β lactam with more potent effects on glutamate did not help. We have not been able to confirm the diagnoses, nor the improvements described in the case series of 3 PALS taking PNG/HC; however, even if these treatment effects were real, these improvements were transient and quickly became unresponsive to treatment. Unless the ongoing placebo-controlled trial shows objective and sustained clinical improvements, we do not recommend that PALS take this expensive and risky combination of penicillin G and hydrocortisone.

Declaration of interest

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