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Treating Neurodegenerative Diseases with Antibiotics

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It is difficult to overstate the impact of penicillin and the family of β-lactam antibiotics since their introduction into clinical medicine in the early 1940s. These drugs act by inhibiting assembly of the protective outer wall of bacteria. Their impact on the treatment of a wide variety of infections has been nothing short of miraculous. But this family of wonder drugs from the last century may have yet more untapped therapeutic potential, as Rothstein and colleagues report in a recent issue of *Nature* (1). They demonstrate that certain β-lactam antibiotics have potential as neurotherapeutics for treating neurological diseases such as amyotrophic lateral sclerosis (ALS), adult motor neuron disease, and ischemic injury.

The evidence for this remarkable finding has arisen from a unique public-private partnership between the National Institute of Neurologic Disorders and Stroke of the NIH and a consortium of disease-oriented philanthropic organizations, including the ALS Association, the Huntington’s Disease Society of America, and the Hereditary Disease Foundation. This consortium sponsored a drug screening effort that ignored the hundreds of thousands of compounds within the traditional chemical libraries mined by pharmaceutical companies. Instead, the consortium screened 1040 bioactive compounds, 750 of which were already approved by the FDA for use in humans. These compounds were then tested for their efficacy in multiple assays associated with one or more neurological diseases by 27 separate academic laboratories.

The first insight to emerge from this approach (see the figure, panel A) was a surprising new function for 15 β-lactam antibiotics, including penicillin and a more modern variant, ceftriaxone, that enters the brain by crossing the blood-brain barrier. These β-lactam antibiotics selectively induce transcription of the gene encoding the EAAT2 glutamate transporter; other classes of antibiotics do not have these effects. Glutamate is crucial for normal signal transmission between many types of neurons, including the motor neurons whose job is to trigger muscle contraction and whose premature death produces the progressive paralysis characteristic of ALS. Upper motor neurons extend processes from the brain into the spinal cord, where they form synaptic attachments directly with the lower motor neurons or indirectly through intermediate neurons. The axonal processes of the lower motor neurons extend out of the spinal cord and form connections with muscle. These neurons communicate with each other by release from the presynaptic cell of the neurotransmitter glutamate (see the figure, panel B), which then binds to receptors expressed by the lower motor neuron. Glutamate receptor activation triggers local membrane depolarization and generation of an electrical impulse that propagates down the full length of the neuron, where it stimulates release of another neurotransmitter that provokes contraction of the muscle. In the spinal cord, a non-neuronal supporting cell, the astrocyte, provides a key element in this signaling pathway—that is, a rapid off switch for the glutamate signal. It does this by juxtaposing a fingerlike projection adjacent to the synapse between the two motor neurons. On the surface of this projection are EAAT2 glutamate transporters, which are glutamate pumps that allow efficient recovery of released glutamate and hence rapid silencing of the glutamate signal.

Excessive glutamate levels in the synapse and associated repetitive firing of neurons results in excitotoxic injury to neurons, a fea-

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**Figure A** Rapid drug discovery by subjecting compounds already approved by the FDA to a battery of new biological screening assays. **Figure B** Communication between motor neurons via the excitatory neurotransmitter glutamate. Glutamate released from the ending of one motor neuron binds to glutamate receptors expressed on the surface of a downstream motor neuron, triggering its activation. Excitotoxic damage results from excessive firing from these receptors, leading to the continued influx of calcium ions and resulting in neuronal injury. The glutamate transporter protein EAAT2 recovers synaptic glutamate such that glutamate neurotransmission is rapidly silenced and the neurons are protected from excess stimulation. Certain β-lactam antibiotics induce expression of the glutamate transporter, reducing the risk of excitotoxic damage to neurons.
tecture of many neurological disorders including stroke, spinal cord injury, and ALS (2). Excitotoxicity is one of the best links between the rare familial form of ALS (caused by mutations in the gene encoding superoxide dismutase) and the more common sporadic form of this disease (3). This realization came from studies in the early 1990s that showed increased glutamate in the fluid surrounding the brain and spinal cord of patients with sporadic ALS (4, 5). Similarly, in a rat model of familial ALS, animals develop focal loss of the EAAT2 glutamate transporter in regions of the spinal cord that house motor neurons (6). Indeed, the only approved medication for treating ALS, the drug riluzole, is thought to act by limiting synaptic glutamate release. The effectiveness of this drug, however, has been disappointing, extending survival of ALS patients by a mere 3 months (7).

The remarkable discovery by Rothstein and co-workers offers renewed hope for a more effective therapy for ALS and other neurological diseases. A ceftriaxone-induced increase in expression of glutamate transporters by astrocytes enhanced the clearance of glutamate in spinal cord explants and, more important, slowed loss of muscle strength and modestly extended survival of ALS mice. Any benefit from increased glutamate clearance also extends to other types of neuronal damage with an excitotoxic component—for example, the damage that accompanies decreased blood flow typical of stroke (frequently referred to as ischemia). A ceftriaxone-mediated increase in glutamate clearance also decreased neuronal death induced by oxygen deprivation, at least in cell culture. The encouraging preclinical data from the ALS mouse and other models of neurological disease have prompted a clinical trial combining all three phases. This is expected to begin this spring with a safety and efficacy study of ceftriaxone in the treatment of ALS. The data generated by the consortium represent a phenomenally quick turnaround from initial drug screening (started in early 2002) to actual use in patients. This reflects the major advantage of screening FDA-approved drugs whose safety profiles are already known. Even more encouraging for the Rothstein et al. findings are the excellent safety profiles of the β-lactam antibiotics in humans. Drug toxicity is costly and time-consuming to exclude and, in the end, is often the Achilles’ heel that sinks the development of promising new therapeutics. In addition, spotting unwanted side effects is challenging even after undertaking multiple preclinical and clinical trials. This lesson was learned most recently with the realization of the increased risk of stroke and heart attack in people taking commonly prescribed anti-inflammatory drugs (8). Against this backdrop are the β-lactam antibiotics, first identified with the discovery of penicillin in 1928 and now among the most widely used modern pharmaceuticals. Although data about the safety of long-term ceftriaxone use still need to be collected, the best predictor of safety is a long history of safe use in humans. Our vast experience with short-term β-lactam antibiotic treatment predicts that very few problems should arise over the long term.

The discovery of new modes of action for the β-lactam antibiotic family offers two additional lessons for biomedical researchers. The first is unproven but predictable: A systematic screen of easily accessible chemical compounds already approved by the FDA may reveal common therapeutics with new potential applications. The second is more surprising: Some of these compounds may act by transcriptional induction of key proteins. Searching for transcriptional up-regulation is not an approach generally thought attractive in drug screening. With that in mind, last century’s miracle drug, the β-lactams, may well rise to one of the big challenges of this century: slowing the progression of neurological diseases whose treatment has so far evaded the world’s best efforts.

References

The Boon and Bane of Radiocarbon Dating
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Radiocarbon (14C) dating (1, 2) is widely used to determine the ages of samples that are less than about 50,000 years old. Natural radiocarbon is mainly formed in Earth’s stratosphere through the interaction of neutrons produced by cosmic rays with 14-nitrogen. However, the rate of radiocarbon production is not constant (3), nor is its partitioning among the atmosphere, terrestrial biosphere, and oceans. After local corrections [see, for example, (4–6)], radiocarbon ages must therefore be calibrated to obtain ages on an absolute time scale (7). For decades, the radiocarbon community has adopted international calibration standards, most recently IntCal98 (8). Here, we discuss the inherent limitations faced when using radiocarbon dates to derive calendar ages.

From modern day to 11,800 years ago, IntCal98 is based on sets of tree-ring chronologies that each cover several thousand years and together provide an annually resolved, nearly absolute time frame. These data set a quality standard against which other proposed calibration datasets can be judged. Prior to 11,800 years ago, IntCal98 is based on marine data and contains additional assumptions and uncertainties associated with the translation of marine data into atmospheric radiocarbon values.

Here we examine how precisely calendar ages can be determined from individual radiocarbon dates. We focus on the tree-ring section of the IntCal98 calibration curve. Between 0 and 8000 years before the present (B.P.), the error in this curve is often less than 20 years, and—except for a few brief intervals—it is less than 30 years over the past 11,800 years. But as we will show, the range of statistically possible calendar ages, or calibrated age ranges, corresponding to any particular radiocarbon date can be larger or smaller, depending on where it falls on the curve.

We have linearly interpolated the IntCal98 curve at intervals of 20 calendar years and determined the radiocarbon dates that correspond to the calendar ages. We then calibrated these resampled radiocarbon ages using CALIB v4.4 (4) assuming an uncertainty of ±40 radiocarbon years, which is currently typical of routine dating (calibration 1). We performed a second calibration with a constant uncertainty of ±15 radiocarbon years, which is typical of the IntCal98 tree-ring data (calibration 2).

The calibrated age range waxes and wanes (see the first figure) as a result of variations in the atmospheric 14C/12C ratio. On average, the 1σ calibrated age range is 180 years (minimum 30 years, maximum 529 years) for calibration 1 and 140 years for calibration 2.