NEURODEGENERATIVE DISEASES

As one door closes, another opens

Each day, about fifteen people are diagnosed with amyotrophic lateral sclerosis (ALS) in the United States alone. Unfortunately, the size of the pharmaceutical armamentarium that is presently available for the treatment of this fatal neurodegenerative disease in no way reflects that of the patient population. Riluzole — a glutamate antagonist — is the only FDA-approved drug that ameliorates the progression of ALS, and it is often used in conjunction with the muscle-strengthening dietary supplement creatine. Two new papers showcase important data on the effectiveness of these compounds in the treatment of ALS.

The widespread use of creatine by ALS sufferers is based largely on results from a mouse model of the disease in which human mutated superoxide dismutase (SOD) is overexpressed. Creatine monohydrate protects motor neurons against degeneration and significantly prolongs survival of these mutants. But the effect of creatine supplementation on ALS progression in humans had not been evaluated until a Dutch consortium published the results of their double-blind, placebo-controlled sequential clinical trial in a recent issue of the *Annals of Neurology*.

Disappointingly, the group found no significant effect of creatine on survival or on the rate of functional decline of riluzole-treated patients. This outcome indicates that the pathological mechanisms of ALS might be fundamentally different in mouse models and affected humans.

Nevertheless, the murine SOD1 mutant model of ALS is one of the best available at present, and new hopes have been raised following the discovery by Jasna Kriz and colleagues that a combination of three drugs substantially delays the onset of symptoms and increases average longevity in this model. Working on the hypothesis that multiple pathways contribute to ALS pathogenesis, Kriz *et al.* targeted three distinct putative pathological mechanisms — the

VIRTUAL SCREENING

Artificial intelligence

Advances in automation and bioanalytical methods have had a large impact on high-throughput screening (HTS), but the number of compounds that can be screened is still small compared with the huge number of possible molecular structures that might be drug-like molecules. As we strive to do things faster and better, the computer continues to reveal how it might play a greater part in HTS. In the 10 June issue of *Proceedings of the National Academy of Sciences*, Schapira *et al.* present an example of the power of 'virtual screening' by using a computer algorithm to develop new thyroid hormone receptor (TR) antagonists for use in hyperthyroidism, when only a related receptor structure is available.

Hyperthyroidism — the overproduction of thyroid hormone — is an extremely common clinical condition, and treatment has remained unchanged for the past 30 years. Therapies include the use of radioactive iodine, surgery or the drug propylthiouracil, which inhibits thyroid hormone synthesis. None of these options are entirely satisfactory, and direct antagonism of the hormone at the receptor level could offer significant improvement for hyperthyroid patients.

Although the structure of TR bound to its natural agonist triiodothyronine is known, antagonists of TR are believed to bind to TR in a different but related conformation. The authors created a hypothetical model of the antagonist-bound form of TR by structural analogy to the ligand-binding domain of the known antagonist-bound structure of the related hormone receptor retinoic acid oestrogen receptor-α. This model was used to virtually screen drug-like molecules as TR antagonists from a library of more than 250,000 compounds. Of 75 molecules that were purchased from the library, 14 showed low micromolar antagonist activity, a far greater number than would be expected from random-based screening methods that only consider the structure of known ligands. The authors then generated a small virtual library of compounds derived from one of the higher affinity antagonists, and a second round of virtual screening identified new compounds with predicted increased antagonist activity. After synthesis, the ability of the compounds to act as TR antagonists was confirmed by transfection and receptor-binding experiments.

These results demonstrate that structure-based virtual screening provides a rapid and powerful tool for the discovery of new bioactive pharmacophores of great structural diversity, and also has utility in the process of lead optimization.

Melanie Brazil

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release of proinflammatory molecules from activated microglia, glutamate-mediated excitotoxicity and increased intracellular calcium concentrations — using co-administration of minocycline, riluzole and nimodipine, respectively.

As minocycline and nimodipine are already approved for other conditions, any potential benefits of their combination with riluzole could be assessed in ALS patients relatively rapidly. Hopefully, this three-drug cocktail will not suffer the same fate as creatine in the transition from mouse to man.

Suzanne Farley

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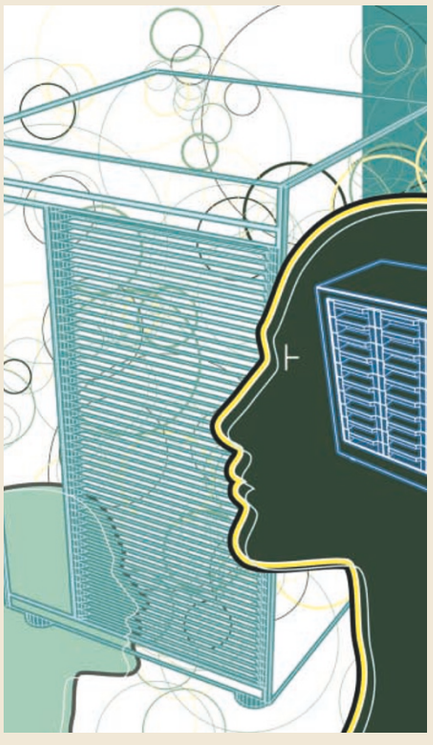
Motor neuron diseases



ANTIFUNGALS

Doing the two step

People with compromised immune systems, such as patients with cancer or AIDS, are often susceptible to severe fungal infections that require long-term treatment. Unfortunately, prolonged use of conventional antifungal agents, such as azoles, can lead to the fungus becoming resistant to therapy. However, a potential solution to this problem has now emerged from a surprising source.

The sodium-channel blocker amiodarone is already approved by the US FDA to treat severe disturbances in the heart's natural rhythm, known as arrhythmias. Last year, researchers investigating its mechanism of action in yeast cells unexpectedly discovered amiodarone's antifungal ability. Now, a study in the *Journal of Biological Chemistry* outlines the two-step mechanism by which amiodarone disrupts yeast cells, and shows that combining the heart drug with azoles provides an unexpectedly effective means of eliminating fungi.

Rajini Rao and colleagues used the baker's yeast *Saccharomyces cerevisiae* as a model to investigate amiodarone's toxicity. As previous work had indicated that amiodarone administration evoked changes in cellular calcium concentrations, the researchers decided to analyse its effect in yeast cells lacking key Ca^{2+} transporters, including pumps, channels and exchangers. Some of these mutants proved to be particularly sensitive to amiodarone, which hinted that Ca^{2+} transport might be important in mediating the toxic effects of the drug.

To further investigate this, a luminescence assay was used to monitor changes in intracellular Ca^{2+} concentrations in response to amiodarone. A two-step response to amiodarone was seen, in which a sharp initial peak in cytosolic Ca^{2+} was followed by a more gradual rise in Ca^{2+} concentrations. Interestingly, mutant cells with higher sensitivity to amiodarone showed sharper rises in cytosolic calcium, further supporting a role for calcium in the amiodarone response.

But where does this amiodarone-induced calcium come from? The researchers showed that amiodarone administration initially causes calcium to flow into the cell from the outside, followed by a more gradual release from internal stores, including the vacuole. They suggest that it is this second sustained rise in calcium that eventually becomes toxic to amiodarone-treated cells. The mechanism by which amiodarone causes cell death by disrupting calcium homeostasis is entirely different to the mechanism used by conventional azole antifungals, which slow cell growth by targeting the ergosterol biosynthetic pathway.

In humans, high doses or prolonged treatment with amiodarone can cause serious side effects, including damage to the lungs and thyroid. The dose required for amiodarone alone to combat fungi is therefore likely to be toxic. Rao and colleagues reasoned that because of their differing mechanisms of action, supplementing an azole with amiodarone could create an effective antifungal treatment, while at the same time preventing the development of resistance. Indeed, they showed that a combination of low-dose amiodarone and the azole miconazole cut survival of yeast cells to less than 10%, compared with almost 100% survival in the presence of the same concentrations of either drug alone. Similar findings were shown with the pathogenic yeasts *Candida albicans* and *Cryptococcus neoformans*, for which amiodarone combined with fluconazole killed almost 100% of cells.

Combining amiodarone with an azole could therefore provide a promising new way of treating fungal infections in individuals with depressed immune systems. Furthermore, this new information about amiodarone's mechanism could help researchers to explain, and eventually prevent, its side effects when used to treat heart problems.

Clare Ellis

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