Modified Curcumin (*Haldi*): A hope in preventing brain cell death in Parkinson's disease

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Behind the screens

See that man, his hands are shaky. When he walks his legs go in a direction he does not intend to. He sits and freezes, stands and pauses. Confused and troubled by lack of co-ordination and stiffness, he undergoes a medical analysis and is alarmed to be diagnosed with Parkinson's disease (PD). If you have witnessed someone dear to you with PD, you will be able to relate. Among many noted personalities, the famous American boxer, Mohammad Ali who knocked many in the ring was himself knocked down by this disease to which he succumbed in 2016. These symptoms of a lifelong debilitating disorder are just the tip of an iceberg. Apart from affecting gait, it disturbs sleep, speech and memory too. It's a paradox that even with ongoing superlative research, there is neither a confirmatory easy diagnosis nor a sure cure of the disease. All the therapies just provide symptomatic relief.

PD is a complex ailment with multiple factors and manifold consequences. Broadly speaking, what we see as a movement disorder is actually a brain degenerative disease in which brain cells of a particular area (*substantia nigra*) responsible for controlling movement start dying. The level of one of the chemicals that transmits signals to the brain (neurotransmitters) called dopamine drops, producing the peculiar symptoms associated with PD.

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Backdrop

Do you know that our body comprises numerous proteins whose functions depend on their structure? Any deviation from the native structure leads to misfolding and aggregation of proteins into soluble and insoluble chunks (*oligomers and fibrils*). There is a quality control system to take care of this and its failure plays havoc as we see in PD. If you postmortem a PD brain, you will find aggregated lumps of proteins. Ever wondered what the key constituent in all these could be? It is a protein called alpha-synuclein. This is an important protein that plays a vital role in not only our brain but other body organs too. It is found to be misfolded and aggregated in a typical PD brain. Although there are a number of environmental and genetic risk factors involved behind the screens but alpha-synuclein is involved in majority of the cases. Hence "synucleinopathies" is the common name given to all the clinical conditions with alpha-synuclein as a key player. Visualise, and it appears that insoluble fibrils deposited in specific brain cells can create a mechanical stress leading to cell death. For many years scientists also believed that it is only the fibrils that are notorious.

But behold! The concept has undergone a paradigm shift. Now most of the neuroscientists have confirmed that it is actually the soluble aggregates of protein that cause more harm through various other mechanisms!

Despite all the efforts, we still lag behind to find a cure. The question which still remains is how to cure the disease? Should we lose hope? Of course not and that is what my research was all about.

> *"There are no such things as incurable; there are only things for which man has not found a cure."*
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> *– Bernard Baruch*

If the disease is looked from a deeper perspective, it appears clearly that resolving protein aggregation would be a better alternative. There is a dearth of valuable molecules that modulate aggregation and the search is still on.

With a determination to seek potential agents that can break the aggregates, I geared up my search for inhibitors which falls into many classes.

- Some inhibitors are proteins that prevent misfolding (*chaperones)*,
- Others are protein-like in nature (*peptidic inhibitors)*.

  Both appear very promising, but they come with drawbacks of
  - Being costly
  - Getting degraded easily.
- Not being able to cross the protection machinery of brain, the blood-brain barrier (BBB)

There is then another vast group of chemical inhibitors (small-molecule inhibitors). This group suffers from a problem of specificity. By specificity, I mean if you intend to target a particular process, these molecules can affect other processes as well. Nevertheless, their superiority in not getting degraded, crossing the BBB, ease of use and cost-effectiveness makes them enticing. So, a

word of caution is to understand the aggregation process elaborately and monitor the efficacy of any of these inhibitors very specifically, intensively and extensively.

Setting the stage

When I set out to design the strategy, one way was to build new molecules altogether and screen them. Another attractive option was to improve upon the existing molecules and study their effects in great detail.

> *"The most fruitful basis for the discovery of a new drug is to start with an old drug."*
> *–Sir James Black*

Every Indian household is familiar with the age-old spice *haldi* (*curcumin*) used extensively in cooking. This small molecule is a wonder molecule with excellent anti-carcinogenic, anti-microbial and even anti-aggregation properties exploited against numerous diseases. The best quality about curcumin is its safety profile. As every good thing comes with a price, curcumin too is marred with limitations. The greatest drawback with curcumin is its instability accompanied with poor water solubility and bioavailability. Nevertheless, I could not overlook the benefits of "modified curcumin", which possesses improved efficacy over normal curcumin.

The first question asked was, Can we have more stable curcumin derivatives?

We were fortunate to have an organic chemist in the lab who synthesized known stable derivatives *viz* curcumin isoxazole and curcumin pyrazole for initial screening

It will be interesting for you to know the highlights of the work comprehensively.

The prelude

Getting the protein

For every protein there is a gene which codes for its synthesis, Alpha-synuclein gene (SNCA) was exploited to synthesize and purify the protein using standardized bacterial machineries which are routinely used for such purposes.

Standardising aggregation

Once I got the purified synuclein, aggregation was studied in different conditions and time periods. To monitor aggregation, I used a chemical named Thioflavin T(ThT). It has an interesting property to fluoresce (emit light) on binding with aggregates which was utilized to standardised aggregation. After several trials, a 30-day window was chosen for further experiments.

Preliminary Screening

Next, I performed a preliminary screen as to which is a better inhibitor; curcumin isoxazole or curcumin pyrazole. Using unmodified curcumin as control, I found curcumin pyrazole to be superior.

Moving a step ahead

Sixteen different pyrazole derivatives were synthesized and screened. Results led to the eureka moment of identification of 3 lead compounds

- compound 3 (curcumin pyrazole),
- compound 6 (N-3-Fluoro phenylpyrazole curcumin) and
- compound 15 (N-3- Nitrophenylpyrazole curcumin).

Long story cut short

With the lead compounds in hand, the next obvious step was to delve deeper to understand the usefulness of the compounds. Till you confirm through many ways, no surety is guaranteed. Hence, I performed detailed analyses of these compounds by various known methods to answer some important questions

What the nature and characterstics are, of aggregates formed in the presence of compounds?

Do the compounds disrupt already formed aggregates?

Are the aggregates soluble?

Are they toxic?

Congo Red is a dye which allows easy visualisation of the presence of fibrils. Samples containing compounds 3, 6 and 15 showed less fibril. These three compounds even reduced the amount of fibrils when added to pre-aggregated proteins.

"Seeing is believing". Advanced techniques like atomic force and transmission electron microscopy helped to directly observe the aggregates. It was again confirmed that compounds 3, 6 and 15 not only inhibited formation of fibrils but also disrupted pre-existing fibrils.

The Finale

All said and done, the biggest question was still left to answer. What is the nature of oligomers formed? Are they toxic and harmful?

For this we used two methods. We cultured brain cells and studied their survival using a chemical called MTT in the presence of aggregates and compounds. We also directly studied the toxicity of oligomers by a known dot blot assay.

Strange and interesting! Compound 6 favoured the formation of toxic end-products rendering it useless as a therapeutic molecule!!

Only compounds 3 and 15 were effective in alleviating toxicity. Compounds 3, 6 and 15 were also assessed for their ability to inhibit the aggregation of another faster aggregating variant of synuclein (A53T mutant). Again, compounds 3 and 15 showed promising results whereas compound 6 generated toxic oligomers.

Take home message and a word of caution

See the irony! Compound 6 which seemed therapeutic via conventional techniques did not impart any beneficial effects in reducing toxicity. Hence, any strategy to discover new molecules should use different methods available and assess toxicity carefully.

Interestingly and pleasingly to conclude, we established that compound 3 and its derivate, compound 15 are potent therapeutic small molecules that can be taken a step further. This step hopefully will move ahead as we intend and not as past American President George W Bush, who struggles with a form of Parkinson's, says "My legs don't move when my brain tells them to."