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Fusogens: chemical agents that can rapidly restore function after nerve injury

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ABSTRACT

Background: Restoring function after nerve injury remains one of medicine's greatest challenges. The current approach of epineurial coaptation does not address the fundamental insult at the molecular level: a discontinuity in the axonal membranes. Membrane fusion is possible through agents collectively called chemical fusogens, which are heterogeneous in structure and mechanism of action. We sought a unifying system for classifying fusogens to better understand their role in cell fusion.

Materials and methods: We conducted a comprehensive literature review to identify the most commonly cited chemical fusogens, their structures, mechanisms of actions, and clinical applications to date. We identified seven chemical fusogens (polyethylene glycol, chitosan, dextran sulfate, n-nonyl bromide, calcium, sodium nitrate, and H-α-7), which have each been studied to different extents in protoplasts, animals, and humans.

Results: Chemical fusogens achieve cell fusion by one of two ways: bringing cells in close enough proximity to each other so the inherent fluidity of the phospholipid membrane allows for their rearrangement or modifying the surface charges of the membranes to diminish repellent charges. Sowers initially put forth a classification system that identified these agents as cell aggregators and membrane modifiers, respectively. We adapted this classification system in the setting of axonal membrane fusion and hypothesized that the most effective approach to axonal membrane repair is likely combination of both.

Conclusions: Chemical fusogens could be grouped into two mechanistic categories—cell aggregators and membrane modifiers. For axonal membrane fusion, a combination of both mechanisms can significantly contribute to advancing outcomes in peripheral nerve repair via a chemical-surgical intervention.

Nerve repair

Nerves, which serve as the wiring of the body, transmit electrical and chemical signals to and from the central nervous system so that the organism can purposefully and safely interact with the outside world. Given the vital role that they play, the ability to repair nerves after they have been injured or otherwise rendered dysfunctional would be immensely valuable. Unfortunately, the ability to reliably repair nerves remains one of the medicine's greatest shortcomings.

The current clinical approach to nerve repair is to coapt the cut ends with sutures in the epineurium. This is largely

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unchanged from the description of a nerve repair technique by Ferrara in the 17th century.[1] Given the lack of clinically effective innovation over the past 4 centuries, it is not surprising that the outcomes after this form of nerve repair are poor. Function (either motor or sensation) is rarely restored to the preinjury state, and the time line of that limited recovery is on the order of years. Furthermore, it is not uncommon to develop anomalous function, in the form of synkinesis, spasticity, or painful neuroma.

The fundamental flaw in the current approach to nerve repair is that it fails to address the defect on the appropriate scale. The functional unit of the nerve is the axon, which is a membranous extension of a single cell (neuron). Each nerve is a bundle of axons (in some cases over 1,000,000) that travel from the spinal cord to the end organ. The axons are grouped in progressively larger bundles that are ensheathed by connective tissue, until the entire nerve is ensheathed within epineurium. While the current repair technique brings two cut ends into approximation and reattaches the ends at the level of the epineurium, it unfortunately does nothing to address the problem at the cellular level. De Medinaceli, who performed pioneering work on advanced nerve repair techniques in the 1980s, referred to this flawed approach as a "tissue-level solution" to what was in fact a "cellular problem."[2,3]

For this reason, a more effective approach to nerve repair would focus on reestablishing membranous continuity at the level of the axon. While this technique is more complex and challenging than the simple epineurial repair, the knowledge and technology necessary to achieve this goal has existed for 40 y and is just now being applied to this critical clinical issue.

Cell fusion

In its strictest definition, cell fusion is the process by which the membranes of two separate cells fuse, thereby combining their intracellular organelles and becoming a hybrid of the two original cells.[4] More generally, it can be considered the process by which any two membrane-bound entities combine into a single entity (e.g., the incorporation of extracellular vesicles into an intact cell).

This is increasingly being recognized as a naturally occurring process, as it is the process by which fertilization occurs, as well as the means by which viruses infect host cells.[5,6] More recently, this process has been harnessed and purposefully initiated; in this case, it is referred to as "cellular engineering," "hybridization," or "induced cell fusion". This was first reported in plants in 1974 and then in animals 1 y later.[7,8] Induced cell fusion has been used for therapeutic purposes such as for the production of monoclonal antibodies (from immortalized B cells known as "hybridomas" that are the result of fusion of a B-cell and a myeloma cell) and most forms of *in vitro* fertilization (notably, this includes mitochondrial donation, which is the controversial technique by which an embryo contains DNA from three separate individuals).[9,10] Currently, researchers are attempting to harness cell fusion to create cancer vaccines and to regenerate damaged cells, particularly myocardium.[11,12]

There are multiple methods by which cell fusion can be performed. These include electrical energy, lasers, viruses (such as the Sendai virus), and the use of chemical agents (known as "fusogens").[6] Of these, fusogens have been most commonly used, owing to their safety and ease of use.

Fusogens

A number of different chemical agents that are able to promote cell fusion have been identified (Table 1). These agents are heterogeneous in both structure and function. Sowers classified fusogens into one of two main groups based on their mechanism of cell fusion. The first group induced fusion via cell aggregation and the other via membrane modification. One fusogen was found to function via both mechanisms. (Figure 1).[13]

In cell aggregation, the fusogen overrides the repellent forces of the membrane surface charges so that the cells are physically close enough such that the intrinsic fluidity of the membranes can predominate and cell fusion occurs. Polyethylene glycol (PEG) is the most extensively studied and commonly used fusogen, and it is thought to function in part via cell aggregation. It is a hydrophilic polymer that is commonly used in a number of chemical, biological, medical, and industrial applications. It was the first agent recognized for having fusogenic properties, as it was shown by Kao and Michayuk in 1974 to successfully fuse plant cells (protoplasts), by Ahkong in 1975 to successfully fuse hen erythrocytes with a yeast protoplast, and by Robinson in 1979, to fuse mouse cells.[7,8,14] Part of the mechanism by which PEG induces cell fusion is "depletion-attraction," such that its hydrophilic nature attracts water, thereby removing it from the space between cells and thus bringing cells into exceedingly close apposition. Other fusogens that function via cell aggregation include chitosan (which is a polysaccharide that induces phospholipid aggregation via electrostatic interactions between its cationic amino group and the cell membranes' anionic phosphate groups), dextran sulfate, and n-nonyl bromide (also known as 1-bromononane).

In membrane modification, the plasma membrane charges are directly altered to prevent repulsion due to like surface charges. In addition to "depletion-attraction," PEG is thought to also function in part via membrane modification. PEG has been found to decrease the surface potential of phospholipids in monolayers and directly bind the cell membrane surface at the lipid-head group. In doing this, PEG compromises the membrane stability and allows unpacking of the lipid bilayer. Thus, PEG not only functions in membrane modification but also a beneficial form of membrane destabilization in addition to its aggregation properties. Other agents in this group include calcium (which influences the action of membranous vesicles that function to seal discontinuous membranes), sodium nitrate, and H-alpha-7 (a newly developed chimeric measles virus hemagglutinin).

While the function of fusogens has been known for 40 y, their application for repair of axon membranes did not occur until 1990 when Krause and Bittner first showed successful fusion of myelinated axons in earthworms.[15] It was not until 2000 that axonal cell fusion was performed in nonhuman vertebrates, including rats, guinea pigs, and dogs.[16-18] In each of these vertebrate models, there was evidence of axonal