Removal of cholesterol and arterial plaque using 2-hydroxypropyl-β-cyclodextrin

Cholesterol, arterial plaque, and heart disease

Cholesterol is a lipid that the human body uses for a variety of beneficial purposes, including ensuring the integrity of cell membranes and synthesizing substances necessary for an organism's survival.[1] A variety of cellular processes are involved in ensuring a proper balance of cholesterol in the body. However, some of these processes can become disrupted, leading to hyperlipidemic conditions that lead to the buildup of unstable arterial plaque that leads to heart disease.[2] In addition to cholesterol, unstable arterial plaque also contains several other components, including lipids (cholesterol), oxidized cholesterol, cholesterol crystals (CCs), and foam cells. Thus, finding a way to inhibit or even reverse the buildup of these plaque-forming materials is key to helping prevent heart diseases.

Lipid removal strategies and 2-hydroxypropyl-β-cyclodextrin

Various therapeutic strategies have been developed to prevent and remove arterial plaque, a causal factor in strokes, myocardial infarctions, and angina.[3] Some of these strategies have included the use of naturally-occurring cyclodextrins (CDs), a family of ring-like molecules whose central cavity can house many different therapeutic compounds such as drugs, thus rendering them soluble in water. However, some CDs produce toxic effects, such as methylated cyclodextrins. This was overcome in the 1980s by modifying the chemical structure of these CDs and converting them into hydroxypropylcyclodextrins. This had the added benefit of improving their water solubility, sometimes even providing a three-fold increase in solubility.[4] 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a cyclodextrin derivative that has appeared in numerous FDA-approved products as an excipient and has been used to solubilize drugs by complexing them in its interior cavity.[5] Many studies have investigated whether a strategy similar to drug complexation can be used to remove excess cholesterol and other arterial plaque components from the body (Figure 1).

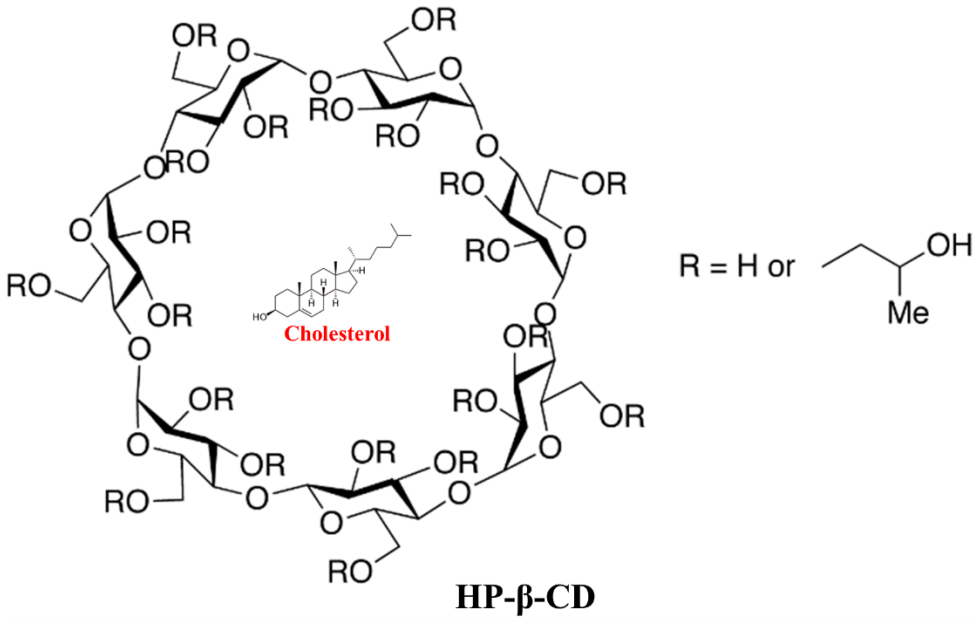

Figure 1 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) can house cholesterol and other plaque components in its interior cavity to eliminate them from the body (*for illustrative purposes only—not to scale*).

2-Hydroxypropyl-β-cyclodextrin and the removal of arterial plaque components

Cholesterol

Decades' worth of research has shown that HP-β-CD can help prevent—and in some studies, even reverse[6,7]—atherosclerosis by removing cholesterol from the human body. Early *in vitro* research investigated the use of a variety of hydroxypropylcyclodextrins, including HP-α-CD, HP-β-CD, and HP-γ-CD, as artificial lipid carriers to supplement the function of natural circulatory lipid carriers. HP-β-CD was shown to better solubilize cholesterol than the other two forms.[8] Therefore, in a companion study, the authors built upon these findings and provided early evidence that the repeated administration of HP-β-CD relieved thoracic aorta lesions in rabbits.[9] In the same study, they also injected HP-β-CD into a human patient and observed a ~20–30% decrease in the total circulating cholesterol.[9] In another early *in vitro* study, Peluso et al. treated HepG2 cells with HP-β-CD and observed rapid efflux of cholesterol from the cells.[10]

Since these early studies, many additional studies have shown that HP-β-CD modifies excess cholesterol efflux from cells to remove it from various parts of the body, including the eyes[11] and brain.[12] The most intense area of research, however, has been investigating its ability to prevent the development of atherosclerotic plaque.[13] Yancey et al. provided kinetics data that showed HP-β-CD provided much more efficient cholesterol efflux than phospholipid acceptors.[14] Another study showed that it was useful for the efflux of cholesterol through the plasma membrane of human cells taken from patients suffering from NP-C.[15] Soon thereafter, other studies showed that it could be used to manipulate the cholesterol content of cells[16] and could

also act as a shuttle that produced a five-fold increase in cholesterol efflux and rapidly cleared both free cholesterol and esterified cholesterol from mouse macrophages.[17] In humans, an *ex vivo* study of carotid plaque showed that HP-β-CD inhibited CC-induced inflammatory responses.[18] Aggregated LDL (agLDL) triggers the retention of cholesterol, which has been shown to promote arteriosclerosis[19]. HP-β-CD was shown to extract free cholesterol from agLDL in a mouse model, potentially helping to reduce cholesterol accumulation.[20]

One of the concerns with high-cholesterol treatment is that patients may not adhere to recommended lifestyle changes to combat high cholesterol. Therefore, it is important to investigate whether the administration of HP-β-CD can cope with cases in which lifestyle changes are not sufficient for reducing cholesterol. Results have shown that both the plaque size and the number of cholesterol crystals decreased in mice given rhodamine-labeled HP-β-CD, indicating that it can help reduce even established plaques.[6,7] It has been shown to decrease lipid accumulation, which reduced the aortic plaque area in male mice.[21] Using lung epithelial cells, Santos et al. showed that a budesonide-HP-β-CD complex showed a greater reduction in cholesterol than either component used alone and also reduced the cytotoxicity of budesonide.[22]

Other plaque components

Many studies in both mouse models and humans have confirmed that HP-β-CD helps remove not just cholesterol, but also other components of arterial plaque, including the oxidation product of cholesterol, 7-ketocholesterol, and sphingolipids. One mouse model study showed that it can accomplish this safely, reducing the size of atherosclerotic plaque (sometimes by up to 45%) without affecting blood pressure, weight, or heart rate.[7]

In the body, low-density lipoprotein (LDL) is oxidized and rapidly uptaken by cells that convert it into lipid-rich foam cells, a component of arterial plaque. During this process, cholesterol can be oxidized into oxysterols, which are known plaque components.[23] The major product of cholesterol oxidation is 7-ketocholesterol, a cytotoxic oxysterol that is linked to various heart diseases.[24,25] Therefore, it is important to also remove this and other oxysterols from the body, which can be accomplished by solubilizing it using HP-β-CD.[26] An early *in vitro* study showed that HP-β-CD removed both 7-ketocholesterol and cholesterol by improving its efflux due to its increased solubility. It showed a preference for removing 7-ketocholesterol vs. cholesterol, but it removed both without inducing cell toxicity.[27] A single high dose of HP-β-CD was shown to reduce the oxysterol concentration in a mouse model.[28]

Glycosphingolipids are another component of aortic plaque,[29] and HP-β-CD has been shown to reduce the amount of glycosphingolipids stored in the body.[30] Various studies have also used HP-β-CD to reduce cholesterol and sphingomyelin accumulation to treat Niemann-Pick disease type C (NPC). In one child, it stopped the disease's progression without showing toxicity.[31] Some sphingolipids are a component of vulnerable arterial plaque and may even contribute to plaque inflammation and the apoptosis of vascular smooth muscle.[32] Therefore, targeting the metabolism and removal of these compounds might be a possible method to treat heart disease. In one study, HP-β-CD was shown to reduce sphingolipids and free cholesterol

accumulation in Npc1-null Chinese hamster ovary cells.[33] In some of the most recent research related to stroke patients, HP-β-CD has been used to reduce lipids, including sphingomyelins, cholesterol ester, and sulfatides,[34] the latter of which has been linked to atheromatous plaques.[35]

Cardioprotective effects

More recent research conducted on rabbits has confirmed that HP-β-CD significantly decreased the area of atherosclerotic lesions and also increased the high-density lipoprotein (HDL) cholesterol levels in plasma, which helps protect against atherosclerosis.[36] These results have suggested that it should also be investigated as a potential therapeutic agent to help treat symptoms in stroke survivors and may even help promote angiogenesis in patients suffering from peripheral artery disease caused by atherosclerosis.[37] In mice, it has also been shown to counteract the effects of obesity—often a comorbidity of heart disease—by helping prevent the intracellular storage of neutral lipids in hepatic tissues and phospholipids in the kidneys.[38]

Mechanism

The ability of HP-β-CD to remove cholesterol is influenced by a variety of factors, including its concentration, incubation time, and temperature.[39] It can also be affected by the structure of HP-β-CD itself, specifically by the number of HP groups it contains, which can range from 1 to 21. A theoretical computational study showed a decrease in its ability to complex both 7-ketocholesterol and cholesterol when more than 9 HP groups were added to HP-β-CD.[40] However, an experimental study showed that the maximum cholesterol solubility was achieved when using 7 HP groups, likely due to a balance between the competing effects of hydrogen bonding (responsible for complex formation) and steric hindrance (which decreases the cavity size).[41]

It is generally accepted that HP-β-CD increases the solubility of cholesterol, which helps the body remove it via the urine, but the exact cellular mechanism by which HP-β-CD normalizes cholesterol homeostasis by removing excess cholesterol from the body is still debated. Several studies have attempted to clarify this mechanism, and recent evidence built upon the findings of Zimmer et al.[7] suggests that HP-β-CD essentially converts the function of cholesterol from inflammatory to anti-inflammatory.[6] Other research has suggested that HP-β-CD changes the expression of some cellular proteins, which suggests that these proteins might be involved in controlling cholesterol homeostasis.[42] It is important to note that it does not appear to remove cholesterol cells with normal cholesterol homeostasis, and appears to only exert a function when excess cholesterol is present (provided the dose of HP-β-CD is not too high or the treatment period is not too long).[10,43] This ensures that it does not remove cholesterol from otherwise normally-functioning cells.

Conclusions

Initially, HP-β-CD was used only as an excipient in drug formulations, but many subsequent studies investigated its ability to remove cholesterol from the body. Almost 30 years of research, conducted in both mouse models and humans, has concluded that HP-β-CD helps the body eliminate several key components of arterial plaque, including cholesterol and its oxidation products, sphingolipids, and foam cells. Work is ongoing to uncover the exact mechanism by which HP-β-CD helps decrease cholesterol levels, but current research clearly shows that HP-β-CD provides a safe and effective method for eliminating arterial plaque components, providing a cardioprotective effect by reducing the buildup of unstable arterial plaque.

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