CYCLODEXTRIN NEWS

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Shift of paradigm on HPBCD as cholesterol sequestering agent in cells

It is well known for the readers of the Cyclodextrin News [1–4] that BCDs, especially methyl BCD and the less toxic HPBCD remove cholesterol accumulated in cells of patients

- with Niemann Pick type C1 disease, where the lack of npc1 and/or npc2 genes responsible for the synthesis of NPC1 and NPC2 proteins transporting cholesterol out of late endosomes cause the lysosomal storage disorder of cholesterol resulting in symptoms of childhood neurodegeneration and these symptoms are improved by HPBCD treatment;
- with cancer, such as leukemia, where HPBCD treatment disturbs cholesterol homeostasis;
- with atherosclerosis, where the cholesterol efflux from atherosclerotic plaques is increased via mobilization of cholesterol with HPBCD;
- with cystic fibrosis, where the malfunction of cholesterol-enriched pulmonary surfactant can be restored by methyl BCD.

The mechanism is not completely understood but it was conceivable to suggest that the high affinity of BCDs to cholesterol is fundamental.

Witkowski et al. [5] reanalyzed existing data of three genome-wide association studies (of 1000-2000 subjects each) on genes involved in breast cancer deaths caused by metastasis. The computational biostatistics approach allowed identification of many genes associated to endo-/exocytosis, and to translocation of **phospholipids** entering the phosphatidylinositol (Fig. 1) cycle. The endo-/exocytosis of oncoproteins, such as growth factor receptors and adhesion molecules, such as integrins and annexins plays a definitive role in progression, migration and invasion of cancer cells. The genetic study showed that downregulation of circulating phospholipids helps to control endo-/exocytosis processes. These novel findings suggest that sequestering phospholipids could be beneficial to control local spread of cancer cells. Based on these results it was hypothesized that the beneficial effect of BCDs is in connection with phospholipid sequestration rather than with removal of cholesterol.

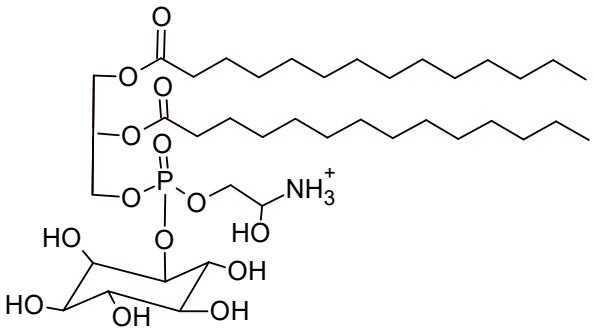

Fig. 1 Chemical structure of phosphatidyl inositol

Various studies –reviewed by Withowski et al.— demonstrated that methyl BCD and HPBCD were found in higher concentration of tumor cells than in the others and were effective in various cancers, including breast, ovarian, lung cancers, melanoma and lymphoma. BCDs showed beneficial effects in several other diseases known to involve disorders in endo-/exocytosis, such as Alzheimer disease, Parkinson disease and atherosclerosis. These effects were earlier explained by initiating cholesterol efflux and the effects on phospholipids were neglected.

On the other hand, Irie & Uekama published in their review on interaction of CDs with cellular membranes as early as in 1999 that not only cholesterol but also phosphatidylcholine and sphingomyelin were removed from cell membranes by BCDs [6]. So, it is a long-known fact that BCDs interact also with phospholipids but this has been overlooked so far.

Witkowski et al. suggest to use alpha-CD (ACD) derivatives, namely **HPACD**, for scavenging phospholipids as a novel therapeutic intervention to control endocytosis and with this the spread of cancer. The ACD and its derivatives are selective for phospholipids, do not interact with cholesterol, and they are less toxic, therefore they have a high therapeutic potential in breast cancer and other diseases.

The other paradigm-shifting topic is that HPBCD-treatment might enhance cholesterol synthesis on the contrary or in addition to the expected effect of cholesterol reduction.

The paper of Gaspar et al. [7] studied the impact of HPBCD on the aging biomarker lipofuscin, which is a yellow-brown pigment, a product of oxidation of low density lipoprotein. Oxidative stress causes significant increases in accumulation of both cholesterol and lipofuscin in cells. The studies showed that HPBCD treatment reduced both cholesterol and lipofuscin in human fibroblasts by reducing *LDLr* and *SREBP1* gene expression of aged cells, but no reduction in cholesterol level was observed in lipofuscin-loaded young cells. It was found that while the cholesterol depletion is beneficial in aged or ill (NPC, tumor, etc.) cells with accumulated cholesterol content of abnormal level, it is harmful in young and healthy cells where depletion of cholesterol from the cell membrane initializes enhanced synthesis and uptake of cholesterol to normalize its level. These two opposite effects depending on the initial cholesterol content of the cells point to the possible harmful effect of HPBCD treatment.

Similar age-dependent effect of methyl BCD treatment was published by Fülöp et al. [8]: methyl BCD induced a significant decrease in the cholesterol content of T cells of elderly subjects whereas it was increased in T cells of young subjects. These results suggest a role for plasma membrane cholesterol in the regulation of the TcR signalling pathways with differential effects related to aging.

A recent paper of Ebner et al. [9] published similar results: liver treatment with HPBCD in a mouse model of NPC1 increased the cholesterol synthesis in addition to decreasing hepatic cholesterol content. This controversy is explained by the enhanced proliferation upon HPBCD treatment and that cholesterol was used for proliferation.

An earlier paper of Liu et al. on studies of HPBCD treatment of NPC1 mice, long-term application of HPBCD in mice raised the *de novo* cholesterol synthesis (lipogenesis) compared to the single application of HPBCD [10].

These exciting findings show that the CDs' effect on living cells is still far from complete understanding.

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Cyclodextrin News Retrospective

We wrote 10, 20 and 30 years ago

10 years ago, the use of CDs in packaging materials was reviewed. Two strategies were compared, a.) when CD complexes are incorporated into the packaging material, b.) when "empty CDs" are present in the packaging films impregnated or grafted. In the first case CD is applied as a tool for controlled release of active compounds when complexes of e.g. antibacterial isothiocyanates, antioxidant a-tocopherol, ripening inhibitor agent 1-methylcyclopropene (1-MCP), dyes or flavors are used. In the second case the CD acts as scavenger for impurities, permeants, or other undesirable volatile contaminants such as plasticizers, aroma components or trichloroanisol.

To exemplify true industrial applicability of this concept, Cellresin technologies offer a patented CD-containing food packaging material containing an effective amount of ripening inhibitor of ethylene ensuring prolonged ripening (i.e. longer shelf-life) of fruit and vegetables (US8414989). Another example is a rodent-repelling trash bag (Mint-X) disclosed in recent patent application (US2017071195) wherein the material of the bag contains essential oil (such as salicylic acid ester, menthol, mint oil, eucalyptus oil, camphor oil) CD complexes. The company promises that trash bags full of kitchen waste waiting overnight till the truck comes to collect them are untouched by hungry cats, dogs, rats, squirrels, opossums, and raccoons.

20 years ago, the topic of the editorial was written on the interaction of natural colorants with cyclodextrins. The article focused on carotenoids and substances of similar chemical structure. CDs, especially RAMEB was shown to increase the aqueous solubility of these guest compounds, wherein the chemical stability of the dyes against thermal decomposition and UV light was improved by alpha-CD in the highest extent.

30 years ago, Cyclodextrin News editorial reported on HPBCD as a newly introduced parenteral excipient and Chiesi's Piroxicam-betadex (Brexin) as the first CD-containing oral drug in Europe. Since then, the solubilizing property of HPBCD has been utilized in vast number of applications, moreover, the potential therapeutic effect of HPBCD alone may offer earlier unimaginable perspectives in medicine. Brexin is still a successfully marketed product. The 25th anniversary of the product introduction was also reported in CD News 5 years ago: https://cyclolab.hu/userfiles/cdn_2013_july.pdf

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Selective attachment of dendrons in the seven positions of the βCD-primary face, CuAAC click reaction, Albendazole

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Mesoporous silica coated gold nanorod, Indocyanine green, End-capping with BCD, Peptide RLA, Reactive oxygen species

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Uremic toxin, Water-soluble adsorbent, Binding mechanism, Removing hydrophobic plasma-bound toxins in dialysis

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Fluoromaltohexaose, Positron emission tomography imaging, Diagnosis of infections associated with implantable cardiac devices

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In vivo myocardic activity, Mice, Contrast agent

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