C60 fullerene with positive effects on immune system

Abstract

Summary and overview of the biological impact, influence and effects of new carbon fullerene on cell health; with the focus on an antioxidant function resulting anti-aging effects combined with life-prolonging. In particular, focused on functions and results of various healing properties and on tested toxic behaviors that have been reported for fullerene and its derivatives in newest publications. The overview starts with the story of fullerene-discovery and the most important properties, functions and effects of carbon fullerenes. This especially with regard of human lifespan extensions due to some antioxidative effects of C60 fullerenes. In comparison with available experimental data and theoretical modeling, a critical consideration and assessment will be developed. Particular attention is paid to a new hypothesis that fullerenes as proton donors (H +) in mitochondria in various simulations of the transport of C60 through lipid bilayer membranes, with their ability to absorb reactive oxygen species. And their degradation activity can act as mild decouplers in mitochondrial respiration. One of the problems that has hitherto complicated the use of fullerene in medicinal chemistry has been its insolubility in water and in water-based liquids. The report created here shows which C60 derivatives nevertheless allow water solubility and how other C60 carriers are used for health-promoting applications. Such carriers are generally fatty glycerol esters or free fatty acids which enable the solubility of C60 fullerene in vegetable oils, namely: olive, sunflower or peanut oils. These results pave the way for use of certain carbon fullerene derivatives in water as well as apply of classic C60 fullerene in vegetable oils as vehicles in the delivery and intake of fullerene for both oral use and vaccine development.

Basics

The first description of the C60 fullerene as hollow carbon molecules was in 1985. Since then there have raised high hopes, particularly with regard to possible applications in the healthcare sector. In the medical-pharmaceutical sector, the use of C60 fullerene is used as an independent medication for the transport (drug carrier) of medication or is also increasingly used in diagnostics as a contrast agent or marker carrier in modern imaging processes. The technical scientific application was awarded the Nobel Prize in Chemistry by chemists from the United States and England in 1996.

These hopes are based on the unique nanometer structure of the carbon molecules and there chemical capabilities, as well as the possibility of different molecule modifications in fullerenes.

Fullerenes often appear very promising in immunology. They are traded as HIV medication, as a suppressor for allergic reactions and as a suppressor in immune-specific cancer therapy.

Therefore it’s of great interest to have more details of fullerenes about effects on the body's immune system: these include the effects of fullerenes on cells of the specific immune defense and their influence on the release of cytokines. In addition, the influence on the different activation of immunocompetent cells is of particular interest for maintaining health.

For the first time, fullerenes were theoretically described and calculated in 1970 by the Japanese Eiji Oosawa, Eiji Oosawa: (Superaromaticity). In: (Kagaku). 25, 1970, pp. 854-863 (Japanese). Since this research was published in Japanese, reference should primarily be made to the first description of Buckminsterfullerene - also called C60 fullerene - by Kroto, Curl and Smalley in 1985 [1].

After graphite and diamond, fullerenes are the third element modification of carbon. The namesake is the American architect Richard Buckminster Fuller, whose constructed domes are very similar to the structure of the C60 fullerenes.
In nature Fullerenes occur only in small quantities as brown-black powder with a metallic sheen. However, they were also found, for example, in shungite coal in Russia. The most common fullerene is the C60 fullerene. It consists of 60 carbon atoms arranged in 12 pentagonal rings and 20 hexagonal rings. Due to the fact that regular pentagons cannot describe a flat plane their spherical arrangement results. They describe a truncated icosahedron, which is often the basis for the production of a soccer ball.

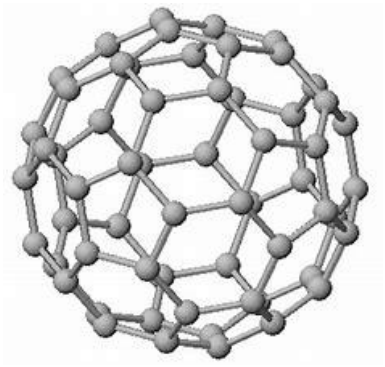

Figure: C60 fullerene
Source: studera.com

The carbon atoms in the C60 fullerene are sp2-hybridized and thus contain aromatic rings and therefore have in particular aromatic properties. Due to the spherical structure and the sp2-hybridized carbon atoms, the fullerenes are strong radical scavengers because they have a large number of electrons in their conjugated double bonds, which led to the designation as a "radical sponge" [2].

Fullerenes can be conjugated exohedral or endohedral, that is, adding molecular groups outside the spherical shape or introducing them inside the sphere [1].

Recently, water-soluble C60 fullerenes have also been used for medical-therapeutic applications. These are fullerenes with the C60 basic structure, which are conjugated exohedral with polar groups. This gives the fullerene polar properties - a formation of separate centers of charge due to charge shift in atomic groups, which cause an atomic group to no longer be electrically neutral - and thus become water-soluble. The resulting electrical dipole moment is a measure of the polarity of a fullerene molecule and thus decisively responsible for the response and capture of charge carriers such as electrons, which are responsible for the damage to the tissue as radicals in the body.

The use of fullerenes in medicine

An important prerequisite for the medical use of nanoparticles such as fullerenes is their influence and also the toxicity on cells in the human body. For every modified form of the nanoparticle, the explicit toxicity has to be recorded. There are several studies and approaches for fullerenes which are not all in agreement [3]. There is an agreement in the statement that the dose makes the poison. Other factors that appear to have an impact on toxicity are: water solubility, contact / incubation time, exposure to light and reactive conjugated groups of molecules.

For example, Sayes et al. [4] described that unmodified C60 fullerenes had a cytotoxic effect on human fibroblasts and astrocytes after 48 hours of incubation, but on the other hand this cytotoxic effect could be reduced or prevented after binding side chains to the carbon framework. Yamawaki and Iwai showed that the concentration of fullerenes has an impact on toxicity using endothelial cells, which they confronted with hydroxy-C60 in a concentration of 100 µg/ml [5]. Lower concentrations had no effect on the cells. Chen et al. also showed that cytotoxicity is concentration dependent. According to their description, oral administration had no effect on the experimental animals in experiments on the rat. [6].

Zhao et al. were able to show on the basis of two fullerenes (C60 and hydroxylated C60) that they produced free oxygen radicals under UV radiation [7]. To do this, they incubated human keratinocytes with the fullerenes and then irradiated them with UV radiation. The pure C60 fullerene was 60 times more phototoxic for human keratinocytes than the hydroxylated C60 fullerene.

The presence of NADH-enzymes (Nicotinamidadenindinukleotid) - more precisely called NADH coenzyme Q oxidoreductase or complex I of the mitochondrial respiratory chain - was required as an electron donor for the formation of oxygen radicals and is therefore an oxidoreductase. In a coupled reaction, the enzyme catalyzes the oxidation of NADH with the reduction of coenzyme Q and combines this with the translocation of protons from the matrix space (inside) into the intermembrane space (outside) of the mitochondrion.

Fullerenes as drugs and drug carriers

There are many ways to use fullerenes in medicine. Fullerenes are suitable, for example, as drug carriers. The drug can be conjugated exohedral or endohedral, or it can be transported in a non-bound manner, especially inside. It is also discussed whether these drug carriers can be made selective for specific tissues using special conjugates. For example, McDevitt et al. a nanotube carrier, which is conjugated with specific antibodies for human lymphoma cells and transports a radiometal ion (thus radiolabeling the protein) [8]. They were able to show that the specified nanotube was very suitable as a drug carrier and that it accumulated well in the target tissue. Furthermore, pharmacological properties of C60 fullerenes are treated per se [9, 10].

Marcorin et al. describe that C60 fullerenes can act as inhibitors of the HIV protease [11]. In this case, the C60 fullerene, with its spherical structure, plays the role of a ligand in the active center of the virus enzyme. However, the fullerene is fixed very loosely. The authors attempted to increase ligand binding by conjugating groups at specific spatial positions of the fullerene that stabilize the binding with the virus enzyme. This interferes or disrupts the virus's maternalization process.

The potential phototoxicity of the fullerenes was described by Kasermann et al. investigated using enveloped viruses [12]. They showed that representatives of the togaviruses and rhabdoviruses, which were swimming in a fullerene solution, lost significantly infectivity (7 log10 / ml) after 5 hours of exposure to visible light. This effect was caused by the formation of free oxygen radicals and was dependent on oxygen.

Influence on cells of the innate immune system

In 2007 Ryan et al. the influence of C60 fullerenes on human mast cells (from the skin and the lungs) and on basophilic cells in peripheral blood [13]. They were able to show that the immune cells responded significantly less to antigen stimulation when they were previously incubated with

fullerenes. The histamine release was also significantly lower, as was the anaphylactic drop in body temperature. According to the result of their work, fullerenes do not prevent antigen-antibody contact on the cell surface, but rather seem to interfere with signal transduction intracellularly. A highly significant inhibition of the phosphorylation of Syk tyrosine kinase was found. Tyrosine-protein kinase SYK, also known as spleen tyrosine kinase, is an enzyme which in humans is encoded by the SYK gene. The phosphorylation of Syk tyrosine kinase correlates with the secretion of mast cells and basophils. With these results, the authors showed that fullerenes can inhibit the immune response of mast cells and basophils. They conclude from this that the fullerenes have immunomodulatory potential in relation to diseases such as asthma, anaphylaxis, arthritis and other autoimmune diseases.

Specific immune response to fullerenes

Chen et al. were able to show in 1998 that the immune system of mice can react specifically to fullerenes [14]. They conjugated C60 fullerenes with bovine thyroglobulin. In mice immunized in this way, IgG antibodies were formed against the conjugated C60 fullerene, which could be detected with the help of an IgG-specific second antibody in the antibody-based detection method ELISA - enzyme-linked immunosorbent assay. These antibodies are cross-reacted with unconjugated C70 fullerenes. However, coupling to a bovine antigen was necessary to sensitize the immune cells to fullerenes. However, the authors have not yet been able to show how the immune system can recognize the fullerenes. They made some hypotheses. The hydrophobicity, curvature, charge distribution and the π bonds play a role here.

Active protection against oxidative stress in peripheral blood mononuclear cell PBMC by fullerenes

PBMC are mononuclear cells of the peripheral blood circulation that have a round cell nucleus. These are, for example, lymphocytes and monocytes. These cells play an important role in the effect of fullerenes on the immune system to fight infections to be considered here.

The postulated property of fullerenes to serve as radical sponges and thus bind free radicals in the event of oxidative stress was developed by Monti et al. examined more closely [15]. It was about the influence of fullerenes on mononuclear cells of the peripheral blood (PBMC), which were to be brought into apoptosis by two different models. The PBMC's were confronted with either 2-deoxy-D-ribose (dRib) or TNF-α plus cyclohexamide. It was recognized that PBM cells that were "protected" with fullerenes showed no changes in the spontaneous apoptosis rate without exposure to dRib or TNF-α plus cyclohexamide. However, the apoptosis rate could be significantly reduced under both trigger models. Monti et al also showed that the percentage of depolarized mitochondria of PBM cells under fullerene protection was significantly lower. This percentage correlates with oxidative stress.

The Monti study describes for the first time the anti-apoptotic effect of C60 fullerenes on PBMC's in oxidative stress.

Report on the current state of knowledge

The scientific knowledge mentioned and cited and the beginning free sale of combination products with olive oil and the associated use of fullerenes for maintaining health inevitably require a more precise determination of the interaction of fullerenes with the human body. It is important to comprehensively clarify the questions of toxicity and the effects on humans or the modulation of

specific tissue in order to ensure safe use and to be able to use any pharmacological effects safely and efficiently.

Since fullerenes represent a relatively new group of substances, there are still too few statements about the interaction with the human body. As mentioned above, modulatory effects have already been described in the area of immune system. However, these were limited to the cells of the innate immune response [13] in vitro or dealt with the provocation of a humoral response of the immune cells to the fullerenes in vitro [14].

Since fullerenes appear to have a modulatory effect on immune-competent cells of the human body, it is of interest to apply this effect to cells of the acquired immune response (B cells, T helper cells and T killer cells), but also in natural killer cells (NK cells) in vitro.

This is particularly so because personalized immunotherapies will increasingly play a major role in future medical-therapeutic applications and will be in the foreground.

A distinction must still be made between the different immune reactions of the TH1 and TH2 patterns, since apparently different cytokines and activation cascades are influenced by fullerenes in both patterns.

Results known so far.

In this overview compiled so far, the effect of fullerenes on cells of the immune system was considered and presented in summary with the help of two representatives as a C60 derivative (polyhydroxy-C60 and N-ethyl-polyamino-C60). The main focus was on the proliferation of PBM cells, the release of specific cytokines and the activation of specific subpopulations such as B cells, T helper cells, T killer cells and NK cells.

Monti et.al. [15] have used methods to determine proliferation using a lymphocyte transformation test, cytokine determination using a sandwich ELISA and measurement of the activation ratio in immune cells with flow cytometry. These are standard test procedures that have long been established in clinical applications. The fullerenes used were purchased in purified form from the manufacturer and provided in a protective atmosphere in solution with RPMI medium. After use, samples of the stock solutions and samples of all concentration levels used were checked with the Endotoxin Detection Test (LAL) for impurities that could lead to unwanted stimulation of the immune cells. This clearly showed that there was no contamination in the stock solutions, the different dilution levels and the media used, i.e. it could be excluded with a high probability that possible reactions of the PBMC are due to unspecific activation by endotoxins.

Useful levels of concentration of fullerenes

Monti et. al. used for the concentration levels of polyhydroxy-C60 fullerene and N-ethyl-polyamino-C60 fullerene from 800 ng / ml to 0.08 ng / ml, which are in the same range of the concentration levels that were also described by Ryan et al. 2007 (1000 - 0.1 ng / ml) which were used to show, for example, the influence of C60 fullerenes on human mast cells and basophils [13].
Preliminary tests have also tested 10 and 100 times higher concentrations, but these did not differ significantly from the 800 ng / ml (in particular, there were no indications of cytotoxicity), so that 800 ng / ml was chosen as the highest concentration level.

Study results of particular interest.

Monti's work does not differentiate whether the entire population of PBM cells proliferates or whether individual subpopulations proliferate. However, the results obtained in his study clearly rule out a negative effect on the proliferation of PBM cells, i.e. the conjugated fullerenes had no cell-toxic effects in selected concentrations. These results from Monti also confirm the study by Dumortier et. al. of 2006, in which modified carbon nanotubes (part of the fullerene family) and their influence on B cells and T cells as well as on macrophages were investigated [16].

Fullerenes only induce increased proliferation in unstimulated cells.

Further results of the investigation of macrophage-typical cytokines IL-6 and TNF-α show that stimulation with fullerenes leads to a significant increase in IL-6 secretion in non-costimulated cells, but not in TNF-α production. When Bacillus Calmette-Guérin (BCG) vaccine was stimulated, there was no significant change in either cytokine secretion. These findings indicate stimulation of the macrophages at rest. However, this effect disappears when the immune system is activated. This proliferation-stimulating effect is therefore many times less than an immune response triggered by antigens.

It can be stated that fullerenes do not lead to a defined cytokine secretion, which favors a TH pattern and would consequently lead to an immune shift through suppression of a TH cell group. In return, the IL-6, presumably secreted by macrophages, could prevent an increased formation of T-regulatory cells, since increased IL-6 concentrations reduce the formation of new T-regulatory cells from naive T cells, according to Fujimoto et al. 2010 [17]. This means that C60 fullerenes could prevent an increased formation of immunosuppressive cells.

Activation of immunocompetent cells under C60 fullerenes.

he protein CD 69 is a surface molecule that is only expressed by activated immune cells. The genes of the activation cascade can be classified according to immediate, earlier and later expression. CD69 belongs to the group of early activation genes (0.5-48 h) and is expressed not only by T lymphocytes [18] but also by NK cells [19] and B cells [20] to the same extent when activated. The CD69 protein is therefore particularly suitable as a marker for the early activation of specific immune cells. There is not necessarily a correlation between CD69 expression and increased proliferation.

Fullerenes therefore have no influence on the activation state in the early phase of an immune reaction and its signaling pathways of the specific immune cells. In contrast, however - as with Ryan et al. Described in 2007 - have an effect on the cells of the non-specific immune defense such as mast cells and - as shown in Ryan's work - NK cells, due to the signal cascade via the Lyn and Syk kinase of the mast cells [13].

The fact that fullerenes are absorbed into immune cells via non-specific endocytosis and are not specifically bound confirms the thesis of non-specific stimulation.

Neither in the work of Dellinger et al. 2010 [21], still in the study by Porter et al. from 2006 [22], whether also deals with the uptake and localization of fullerene inside the cell, describes a receptor presentation of these fullerene particles by MHC molecules.

If one looks at the results, the proliferation, the cytokine secretion and the CD69 expression, the stimulation of the IL-6 secretion and the proliferation in non-costimulated PBM cells are noticeable. However, there is no TH1 / TH2 specific cytokine stimulation. This leads to the consideration whether the observed proliferation increase under C60 fullerene could be an increase in the macrophages

Preliminary consideration

The fact that the fullerenes tested did not show immunosuppression in any study and the fact that only unconjugated fullerenes (Kolosnjaj et al. 2007 [23]) or conjugated fullerenes in significantly higher doses (Yamawaki et al. 2006 [5]) are toxic for cells, rules out an inhibition / weakening of the cellular immune defense by the fullerenes used in the respective studies. This thesis is confirmed by the unchanged cytokine profile and the constant proportion of activated leukocytes.

It is currently not possible to say whether there will be a negative humoral answer. As in 1998 Chen et al. described: Antibodies against fullerenes are possible but the targeted formation of antibodies is only caused by conjugation with a foreign protein (here bovine thyroglobulin) [14]. A stimulation of the TH2-typical cytokines and also an activation of CD19 + B cells have not been observed so far. However, there is no doubt that IL-6 secretion is increased, which both stimulates and produces B cells.

The large group of fullerenes and their derivatives has not yet been developed as a whole. Individual properties, the reactive groups, the concentration and external influences can determine the picture of how fullerenes interact with their environment.

Summary

Since their discovery, fullerenes have raised great hopes for their chemical structure. Fullerenes are increasingly being better understood and used in medical health services. They can assume a variety of roles: be it as a transporter, as a link between the drug and the targeting protein, as a contrast agent or as an independent drug.

Many previous studies have ruled out a generally toxic effect of fullerenes. Several studies describe the effect of fullerenes as a drug or as a contrast agent. However, there is little work (apart from the cell toxicity studies) that deals with the interaction of this group of substances with the human body itself. There are only isolated studies of the immune system.

Due to the limited data and the knowledge that fullerenes seem to have an influence on the innate immune response, it is of great interest to better understand the influence of fullerenes on the body and the immune system.

Reports were included in the studies and dissertations considered and cited as follows:

- The influence of fullerenes on the proliferation of PBM cells,
- The cytokine production using IL-1, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, TNF-β, GM-CSF by means of sandwich ELISA and
- early activation of immune cells using CD69 expression using flow cytometry.

As further result, it could be shown here that

- Fullerene derivatives stimulate the proliferation of PBMC,
- Increased CD69 expression occurs only on NK cells,
- The production of T cell-associated cytokines is without fullerene influence, on the other hand
- Fullerenes significantly increase the secretion of IL-6.

In addition, it was found in reports mentioned that fullerenes have no general immunosuppressive effect and are not cell-toxic and in particular do not significantly affect the cells of the specific immune system.
In contrast, some findings indicate that cells of the innate immune system are activated (e.g. NK cells).

Further reports on the interaction and influence of the C60 fullerenes in specific clinical pictures such as Alzheimer's, rheumatoid arthritis or autoimmune or neurodegenerative diseases are in preparation.

Sources

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