KOPF CARRIER

September 2006

Kopf Carrier #63

PUBLISHED BY DAVID KOPF INSTRUMENTS ■ TUJUNGA, CALIFORNIA

CARDIOVASCULAR EFFECTS OF FULLERENE DERIVATIVES: A REVIEW OF CURRENT EVIDENCE

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Abstract

During two last decades, several unique physical and chemical properties of buckminsterfullerene or fullerene C60 have been described. However, much less is known about the effects of fullerenes and their derivatives on biological systems. Evidence is beginning to accumulate that fullerenes may exert influence on different physiological and pathophysiological processes primarily because of their antioxidant effects. The present paper focuses on the cardiovascular effects of fullerene C60 and its water-soluble derivatives. We present available evidence on the protective effects of fullerenes in ischemia-reperfusion injury and their influence on vascular tone. In addition, we review data on the antiproliferative and antiatherogenic effects of fullerene derivatives. Perspectives of fullerenes utilization for photodynamic therapy of cardiovascular diseases are also discussed. Current findings demonstrate that fullerenes may show several potentially physiologically and clinically relevant activities, including antiischemic effect, vasodilatation, inhibition of low-density lipoprotein oxidation, and limitation of proliferative activity of vascular smooth muscle cells. Additional studies will be required to define the molecular mechanisms responsible for the observed effects.

Key words: fullerene, ischemia-reperfusion injury, oxidative injury, vascular tone, atherogenesis.

Editor's Column

Soon, many of us will be in Atlanta, Georgia for the major event of the Neuroscience year, the Society for Neuroscience Convention. I hope to see many of you there. Please stop by the David Kopf Instrument booth, #1204, to say hi, look at the new displays and chat with any of us about your work. I would also invite anyone who would like to write an article for the Carrier to stop by to talk with me. David Kopf Instruments has sponsored the Carrier since 1972! It is the oldest such newsletter in the industry and has published many very useful articles. There is an honorarium for each article published, and almost any topic is considered. Please look over the back issues also available online.

We also invite you to attend the David Kopf Memorial Lecture on Neuroethics. This centerpiece lecture will be given this year by Judy Elles, PhD of Stanford on Monday, October 16 from 10-11 am. Dr. Elles lecture is titled "Neuroethics, Neurochallenges: A Needs-Based Agenda." The lecture is sponsored by David Kopf Instruments in memory of David Kopf.

I wonder how many of you remember the very first society meeting in Washington, in 1971. There were not so many of us at that convention, only 1396, held in the Shoreham Hotel. (If you want to see the stats of all the meetings, go to www.sfn.org/index.cfm?pagename=annualMeeting_statistics). Of course the society and the meeting has grown considerably since, and obviously is fulfilling a great need in the scientific community. David Kopf Instruments was one of the very first corporate sponsors of the society and through such venues as the Carrier has continued to support the neuroscience community. Of course, the company also supports the science of neuroscience by providing the highest quality and most complete range of stereotaxic instruments in the world.

This edition of the Carrier is from our Russian colleagues, This issue of the Carrier is again from our Russian Colleagues Alexander Syrensky, Elena Egorova, Illia Alexandrov and Michael Galagudza. Their review of cardiovascular effects of Fullerene derivatives is very interesting and enlightening. I commend it to your reading.

Other news from here in Florida; we have just escaped a bullet from nature, hurricane (tropical storm) Ernesto. I think that many people here were much better prepared for a storm this year than last, but all we got out of it was a windy day with a little rain. The university closed for two days on the strength of the forecast that had the track right but the intensity wrong. However, it is much better to be over prepared and not need it than under prepared and get hit hard. It is likely, however that we will have another storm or two this season, so it was a good dry run. I think I have enough mac and cheese and canned chili to last about 3 weeks.

I look forward to seeing you at the Neuroscience meeting. Please stop by to say hello.

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Introduction:
antioxidant effects of fullerene derivatives

The marked ability of fullerene C60 and its derivatives to inactivate reactive oxygen species (ROS) has been described in 1991 by Krustic and co-authors who characterized C60 (buckminsterfullerene) as a «free radical sponge» [1]. Indeed, one molecule of fullerene C60 is capable of adding 34 methyl radicals. Antioxidant efficiency of fullerene depends on the number of active centers in the molecule and the distance between active centers and target atoms. Fullerenes can quench superoxide anion radicals and hydroxyl radicals both in vivo and in vitro.

Fullerene C60 can be readily dissolved in organic solvents but it is practically insoluble in water. Poor solubility in water severely hampers investigation of physiological and pharmacological effects of fullerenes. As a consequence, a number of C60 derivatives with better solubility in polar solvents have been synthesized to date. In particular, one of the new water-soluble C60 derivatives, hexasulfobutyl[60]fullerene (FC4S), contains 6 sulfobutyl groups covalently bound to the Ñ60 frame. Recently, the antioxidant effects of FC4S have been studied in the isolated rat heart model according to Langendorff [2]. FC4S was added to the perfusion fluid, and the hearts were subjected to 15 minutes of global ischemia and 30 minutes of reperfusion. The content of ROS in the coronary effluent samples was determined using electron-spin resonance spectroscopy (ESR). The intensity of ESR signal which correlates with the content of free radicals was significantly lower after addition of FC4S to the perfusate.

Fullerenol-1 represents another water-soluble derivative of fullerene C60 with simple chemical structure consisting of 60 carbon atoms and multiple hydroxyl moieties. Antioxidant activity of fullerenol-1 has been demonstrated on grafts after small intestine transplantation in canine model [3]. It is now established that reperfusion of ischemic graft causes massive generation of ROS which, in turn, play a key role in graft damage. This fact is supported by diverse evidence, including increased content of malondialdehyde (MDA) and conjugated dienes (CD) in the reperfused graft tissue. Intravenous administration of fullerenol-1 caused significant decrease in tissue content of MDA and CD as determined after 30 and 60 minutes of reperfusion. Furthermore, fullerenol-1 preserved graft from associated with ischemia-reperfusion depletion of tissue glutathione stores.

Fullerenes and ischemia-reperfusion injury

Protective effects of fullerenes in ischemia-reperfusion of the lung Ischemia-reperfusion injury of the lung has a complex pathogenesis which includes vascular dysfunction, inflammation, and edema. However, the key role in the pathogenesis of ischemia-reperfusion injury to the lung belongs to oxygen free radicals. There are three major sources of free radicals during ischemia-reperfusion:

1. During reperfusion, oxygen delivery to the tissues is restored, and molecular oxygen initiates the process of xanthine and hypoxanthine oxidation by xanthine oxidase which leads to the formation of excessive amounts of both superoxide anion radical and hydrogen peroxide. Hydrogen peroxide, in turn, is converted into hydroxyl radicals by means of reducing metals, particularly, Cu+ and Fe2+.

2. Damaged during ischemia mitochondria may become a source of electrons due to their «leakage» from electron transport chain. These electrons participate in the generation of superoxide. Tissues damaged by ischemia can produce increased amounts of chemoattractants for neutrophils such as leukotriene Â4 and platelet activating factor. Furthermore, during postischemic reperfusion the endothelial expression of adhesive molecules is increased, and activated and attracted to the site of injury

neutrophils can release additional free radicals. Free radicals cause vasoconstriction, which is a hallmark of ischemia-reperfusion lung injury.

One of the mechanisms whereby free radicals cause lung injury is the interaction between hydroxyl radical and hydrogen atoms of the methyl groups of polyunsaturated fatty acids. This process initiates lipid peroxidation of membrane lipids which, in turn, leads to the increase in cellular membrane fluidity and permeability. It has been long recognized that different types of antioxidants are able to mitigate ischemia-reperfusion lung injury. In the study performed by Lai et al. the protective effects of water-soluble fullerene C60 derivative Ñ60(ONO2)7±2 were investigated in the isolated ischemic-reperfused rat lung [4]. It was shown that Ñ60(ONO2)7±2 possesses antioxidant properties and it can furthermore release nitric oxide displaying the effects similar to those of nitroglycerine. Experimental protocol included 10 minutes of stabilization, 45 minutes of ischemia, and 60 minutes of reperfusion. The lungs were ventilated with gas mixture containing 95% of Î2 and 5% ÑÎ2. The pulmonary arterial (PPA) and venous pressures, lung weight (W), pulmonary capillary pressure, and filtration coefficient (Kfc) were registered both before and after ischemia. Ischemia caused increase in PPA, W, and Kfc in controls, but Ñ60(ONO2)7±2 limited this increase, and this was considered as an alleviation of ischemia-reperfusion lung injury.

Protective effects of fullerenes during intestinal ischemia-reperfusion At present, there is only one published study describing protective effects of fullerenes in intestinal ischemia-reperfusion [5]. In canine model, 60 minutes of small intestinal ischemia were followed by 1 hour of reperfusion. Fullerenol-1 at a dose of 1 mg/kg was administered intravenously 30 minutes prior to ischemia (preventively) and immediately after reperfusion (therapeutically). An increased amount of both MDA and CD was found in the intestinal tissue on the 30th and 60th minutes of reperfusion in control experiments. Tissue content of glutathione, in contrast, was decreased after 60 minutes of reperfusion. Histological changes of small intestine after 60 minutes of reperfusion included slight detachment of the apical villous epithelium and modest mucosal edema. Fullerenol-1 administration did not change histological picture but caused significant decrease in tissue content of MDA and CD and furthermore increased glutathione level in both preventive and therapeutic protocols.

Protective effects of fullerenes in cerebral ischemia-reperfusion One of the most widely used experimental models of focal cerebral ischemia is permanent occlusion of middle cerebral artery (MCA) in Mongolian gerbils via subtemporal craniotomy. The advantage of this technique is that gerbils do not have anastomoses between carotid and vertebrobasilar systems of cerebral circulation which allows getting more reproducible infarct size data after MCA occlusion. With use of this model, Yang and co-authors investigated the influence of FC4S on the ischemic brain injury caused by permanent 24 hour occlusion of MCA in gerbils [6]. Three groups of animals were investigated: controls, low (0.5 mg/kg/day) and high (5.0 mg/kg/day intraperitoneally during 2 weeks) dose of FC4S. After 24 hours of MCA occlusion infarct size was determined with triphenylterazolium chloride staining. Prolonged FC4S therapy resulted in significant decrease in cerebral infarct size (by 42-68% in comparison with controls). The authors suggested that neuroprotective effects of FC4S are secondary to its antioxidant properties. Of note, in this study a moderate toxic effect of FC4S was registered and it manifested as a 10% decrease in animal body weight after 2 weeks of daily intraperitoneal FC4S injections.

Huang and co-authors [7] investigated the effects

of FC4S on cerebral infarct size in Long-Evans rats in vivo. Cerebral infarct was induced with 60 minutes of right MCA occlusion combined with occlusion of both common carotid arteries. Infarct size was determined after 24 hours of ischemia. FC4S was administered intravenously at a four different dosages (0.1, 1, 10, and 100 ìg/kg) in both preventive (15 minutes before MCA occlusion) and therapeutic (after declamping of carotid arteries) regimen. Administration of FC4S in high dosages (10 and 100 ìg/kg) caused significant infarct size limitation in both preventive and therapeutic protocols. Besides, FC4S decreased the level of lactate dehydrogenase in plasma, and this was considered as an additional evidence for neuroprotection. Plasma nitric oxide content after administration of FC4S was, in contrast, increased.

In a recent study by Lin et al. [8], the same rat model of cerebral ischemia was used to evaluate the effects of systemically and locally administered carboxyfullerene. Carboxyfullerene was injected 30 minutes prior to ischemia-reperfusion either intravenously or intracerebroventricularly. Carboxyfullerene did not alter infarct size after intravenous administration, which may be due to limited permeability of blood-brain barrier for this compound. Local administration of carboxyfullerene resulted in infarct size limitation, preservation of tissue stores of glutathione, and decreased amount of lipid peroxidation products in the ischemic brain cortex. Local carboxyfullerene administration caused adverse behavioral reactions in rats, particularly, hyperkinesias accompanied by trunk stretch and even death of 20% of the animals. These data indicate on the potential toxicity of carboxyfullerenes.

Fullerenes and vascular tone

One of the water-soluble derivatives of fullerene Ñ60 is monomalonic acid Ñ60 (MMA Ñ60). MMA Ñ60 causes specific inhibition of acetylcholine-induced relaxation of vascular smooth muscle cells during myography of spiral strips of the rabbit thoracic aorta [9]. Acetylcholine stimulates generation of nitric oxide (NO) in the endothelium and causes relaxation of vascular smooth muscle precontracted by phenylephrine. It follows, therefore, that inhibition of vasodilatation caused by MMA Ñ60 may be potentially due to either blockade of endothelial NO production or acceleration of NO inactivation by ROS, for instance, superoxide. The amplitude of the inhibiting effect of MMA Ñ60 was approximately 59.7% of maximal acetylcholine-induced relaxation. This fact suggests that MMA Ñ60 has the marked ability to generate superoxide [9]. In support of this assumption, it was found that inhibiting effect of MMA Ñ60 on acetylcholine-induced relaxation was abolished by addition of superoxide dismutase (SOD).

MMA Ñ60 also inhibited relaxation of aorta without endothelium caused by NO-generating agent, S-nitroso-N-acetylpenicillamine (SNAP). This inhibiting effect was also attenuated in the presence of SOD. On the other hand, MMA Ñ60 did not attenuate relaxation of aorta caused by sodium nitroprusside, which is another NO donor. It is known that SNAP and sodium nitroprusside release NO by means of different mechanisms, and this may explain the difference in the effect of MMA Ñ60 on SNAP- and sodium nitroprusside-induced relaxation. SNAP produces NO in an enzymatic way, and the enzyme responsible for that is localized on the surface membranes of vascular smooth muscle cells while sodium nitroprusside releases NO in the myocyte cytoplasm and simultaneously activates soluble guanylate cyclase.

Furthermore, MMA Ñ60 had no influence on â-adrenergic agonist-induced relaxation of the guinea pig trachea, acetylcholine- and histamine-induced contraction of ileac smooth muscle, serotonin-induced contraction of the rat stomach (fundus) and phenylephrine-induced contraction

of rat vas deferens. These data are indicative of the fact that MMA Ñ60 can not inhibit agonist-induced contractile responses of the smooth muscle, but it can inhibit NO-mediated relaxation of vascular smooth muscle, probably due to generation of free radicals. Alternative and even more possible explanation for the inhibiting effect of fullerenes and their derivatives on the endothelium-dependent vasodilatation has been proposed by Wolff and co-authors [10]. In this study, the influence of Ñ3- and D3-tris-malonyl-Ñ60-fullerene on the activity of three isoforms of NO-synthase (NOS), an enzyme which catalyzes the production of NO from L-arginine, was investigated. Both Ñ3- and D3-tris-malonyl-Ñ60-fullerene inhibited all three NOS isoforms. Of note, calcium- and calmodulin-dependent constitutive NOS isoforms (neuronal, endothelial) were inhibited by fullerenes to a greater extent than inducible isoform. Ñ3 isomer exhibited more pronounced inhibitory influence on all three isoforms of NOS than D3 isomer. In addition, the inhibition of NOS isoforms with fullerenes was completely reversible. It seems likely that the inhibitory effect of fullerenes on NOS function is not linked to their antioxidant properties but rather is a consequence of their direct interaction with enzymes. For normal function, which includes NO generation and reduction of oxygen, i. e. NADP-oxidase activity, NOS must have a homodimer structure. It was suggested that the binding of fullerene to highly hydrophobic site of NOS molecule located in the region of contact between two subunits reversibly interferes with their normal alignment which is essential for the transport of electrons from one subunit to another.

Huang and co-authors [11], while studying the effects of FC4S and MMA Ñ60 on the intact and denuded of endothelium aortic rings, have not found any relaxing effect of MMA Ñ60 on the precontracted by phenylephrine preparations with intact endothelium. In contrast, acetylcholine caused relaxation in the same conditions, and this relaxation was significantly attenuated by MMA Ñ60. FC4S caused relaxation of precontracted by phenylephrine aortic rings. Acetylcholine-induced relaxation of precontracted by phenylephrine aortic strips was significantly potentiated in the presence of FC4S. Interestingly, FC4S did not cause relaxation on the aortic rings without endothelium. Furthermore, relaxing effect of FC4S was not abolished by administration of SOD. However, it was partially abolished after pretreatment with N(G)-nitro-L-arginine and methylene blue. These observations suggest that vasodilatory effect of FC4S is partially dependent on the release of NO and/or NO-dependent factors from the vascular endothelium. There is a definite possibility that FC4S activates NO release from the endothelium of the aortic rings.

Antiproliferative and antiatherogenic effects of fullerenes

The key pathogenetic features of atherosclerosis and post-angioplasty restenosis are accumulation of extracellular matrix proteins and pathologic proliferation of vascular smooth muscle and inflammatory cells. Therefore, proliferative responses of vascular smooth muscle cells and mononuclear leukocytes play a significant role in the pathogenesis of atherosclerosis and restenosis. It has been shown earlier that some hydroxylated plant phenols exhibit antiproliferative effects in vascular smooth muscle cell cultures [12]. These compounds possess antioxidant properties and they furthermore are able to inhibit tyrosine kinase.

It has been recently demonstrated that fullerenols also reduce proliferative capacity of vascular smooth muscle cells and mononuclear leukocytes. Fullerenol-1 inhibited proliferative responses of vascular smooth muscle cells and mononuclear cells, particularly human lymphocytes and smooth myocytes of rat aorta

(A7r5 cells) and human coronary artery [12]. Furthermore, this effect of fullerenol-1 was stronger then that of ascorbic acid and it was concentration-dependent. Besides, the alloxan-induced superoxide generation in smooth muscle cells was inhibited by fullerenol-1. It has been shown that diabetogenic drug alloxan stimulates superoxide generation which is attenuated by SOD. It seems not unlikely that fullerenol-1 works as a scavenger of free radicals. It is known that superoxide inactivates NO and as a result yields highly reactive peroxynitrate radical (ÎNOO-). On the other hand, SOD prevents inactivation of NO by superoxide. It follows, therefore, that fullrenol-1 may act via inhibition of superoxide thus preventing inactivation of NO and contributing to preservation of vasodilatory reactions and antiatherogenesis.

One of the major factors contributing to the pathogenesis of atherosclerosis is oxidative modification of low-density lipoproteins (LDL). It has been long recognized that oxidatively modified LDL are taken up by the macrophages which leads to the accumulation of lipids and foam cell formation. An increase in the LDL tolerance to oxidation may attenuate and even prevent the development of advanced atherosclerotic lesions. At present, several reports provide the evidence that natural and synthetic antioxidants may attenuate the severity of atherosclerotic lesions in animal models and in humans. In particular, vitamin E or á-tocopherol is considered to be one of the most efficient inhibitors of LDL oxidation mainly because of high solubility in lipids. However, antioxidative potential of vitamin E have been questioned during last years. It has been shown that vitamin E facilitates the transfer of free radical reactions from aqueous to lipid phase and it furthermore mediates chain free radical reactions in the lipoprotein core [13]. In this regard, great efforts are currently applied to develop new effective antioxidants lacking such a prooxidant activity. In the study by Lee et al. the effects of water-soluble Ñ60 derivative (FC4S) on the LDL oxidation in aqueous and lipophilic phases were investigated [14]. FC4S inhibited LDL oxidation in both aqueous and lipophilic phases. Moreover, intravenous infusion of FC4S during 6 weeks significantly reduced the extent of atherosclerotic lesions in the ascending aortas of rabbits fed on high-cholesterol diet.

The mechanisms by which water-soluble antioxidants protect LDL from oxidation include quenching of free radicals in the water phase before their interaction with lipoprotein lipids and/or preservation and regeneration of lipoprotein-bound antioxidants (preventive strategy). Bound to lipoproteins lipophilic antioxidants act as chain-breaking agents and suppress lipid peroxidation by reducing lipid peroxy radicals and formation of covalent links with them. In contrast to water-soluble antioxidants, chain-breaking antioxidants can not prevent the initiation of lipid peroxidation or keep endogenous antioxidants inside lipoproteins. Taking into account these differences of water and lipid soluble antioxidants, the data about high antioxidant activity of FC4S suggest that this compound may work both as a preventive and chain-breaking antioxidant. Although the exact mechanism whereby FC4S protects LDL is not clarified yet one may assume that it involves scavenging of aqueous free radicals owing to easy distribution of FC4S in water. Hydrophilicity of FC4S is explained by its high electronegativity. Alternatively, it may be explained by specific binding of FC4S to LDL. The latter explanation provides clue to the fact that water-soluble FC4S inhibits lipid peroxidation of LDL induced by lipophilic initiator and significantly reduces further peroxidation of lipids even after beginning of the propagation phase.

The results of epidemiological studies show that vitamins with antioxidant properties may prevent atherosclerosis. The content of ascorbic acid in the plasma is inversely related to the mortality

from coronary artery disease. There is some evidence that ascorbic acid inhibits proliferative responses of vascular smooth muscle cells and lymphocytes. However, antiproliferative effects of vitamin C in vitro in the cell cultures are significantly less than that of fullerenol-1. It seems that fullerenol-1 and ascorbic acid apart from intrinsic antioxidative activity are able to possess certain effects on intracellular signaling cascades. It is possible that antiproliferative activity of fullerenol-1 may be mediated by inhibition of signaling pathways leading to DNA synthesis. In particular, fullerenol-1 and ascorbic acid dose-dependently decrease the activity of tyrosine kinase while their influence on protein kinase C seems to be much less pronounced [12]. It follows, therefore, that the inhibition of tyrosine kinase may present one of the mechanisms of antiproliferative effect of fullerenol-1.

Perspectives of fullerenes utilization for photodynamic therapy of cardiovascular diseases

Photodynamic therapy (PDT) is aimed at selective destruction of abnormal, e. g. tumor or hyperplastic tissues (for review, see [15], and references therein). The compounds which are used for PDT are called photosensitisers, and there are two general requirements for such a drugs first, they must be able to accumulate in the pathologic tissue and, second, they should be readily converted to their excited (triplet) state upon irradiation with a visible light of a specific wavelength. The cytotoxic effect of PDT is mediated by a massive generation of singlet oxygen and free radicals which occur as a consequence of interaction of excited photosensitiser and tissue oxygen.

Fullerenes and their derivatives are readily converted into their excited states during ultraviolet and visible irradiation [16]. In turn, the molecules of fullerene in the triplet state can convert tissue oxygen into singlet oxygen (1Î2).

These photochemical properties of fullerenes suggest that they could be used as efficient photosensitisers for PDT [17]. Studies using murine model of tumor growth have generally confirmed that PDT with polyethylene glycol-modified fullerene C60 may exhibit antitumor effects [18].

In the past decades considerable information has been accumulated on the therapeutic applications of PDT in oncology, ophthalmology, and dermatology. However, until now the use of PDT for treatment of cardiovascular disorders has been mainly limited to the attempts to suppress pathologic proliferative activity of endothelial and vascular smooth muscle cells and thus attenuate atherosclerosis and postangioplastic coronary restenosis.

Several reports showed that PDT may inhibit proliferation of smooth muscle cells and intimal hyperplasia. In 1992, Ortu and co-workers found decreased intimal hyperplasia after PDT in the rat model of carotid artery balloon injury [19]. More recently, similar results were obtained by others [20, 21]. PDT is followed by reversible morphologic changes in all three layers of vascular wall, namely endothelial denudation, medial cell depletion, and reduction in number of adventitial myofibroblasts. In the in vitro experiments PDT significantly decreased fibroblast growth factor 2 induced mitogenesis of smooth myocytes [22]. PDT of the bovine endothelial cell culture caused the changes in extracellular matrix (ECM) properties: when separated from the cells after PDT, ECM acquired the potential to inhibit proliferation and migration of smooth myocytes [23]. In contrast, proliferation and migration of endothelial cells was increased after treatment with ECM which underwent PDT. These PDT-induced changes in vascular elements may potentially benefit the process of vascular remodeling and decrease the probability of restenosis.

There is some, but not yet rigorous evidence, that

PDT may be effective in reducing experimentally induced atherosclerotic vascular lesions. For instance, in the rabbit model Litvack and co-authors observed the regression of photosensitized atheromatous plaques in the carotid arteries after irradiation with light [24]. The mechanisms underlying beneficial effects of PDT on atherosclerotic lesions may involve the changes in lipid biochemistry and depletion of cholesterol esters [25].

The effects of PDT on the intact arterial vessels are also known. It has been demonstrated that PDT of normal vessels causes a unique biological response including endothelial denudation and dose-dependent thinning of medial vascular layer due to depletion of vascular smooth muscle cells [26, 27]. Of note, these striking histological changes occur in the absence of local inflammatory reaction. This observation suggests that the major mechanism of PDT-induced vascular cell death is apoptosis [28]. On the other hand, it has been shown that PDT of rat carotids results in the decreased amount of fibroblast growth factor â in the vascular wall [22]; PDT in vitro causes an attenuation of transforming growth factor â1 activation and release [29]. These data provide evidence for the existence of cytokine-modulating effects of PDT. The PDT-induced changes in arterial wall are completely reversible. Reendothelisation of PDT-treated vessels has been reported to be accomplished within 14 days after intervention, while repopulation of smooth muscle cells is more delayed and may last up to 6 months [30].

To the best of our knowledge, the fullerene derivatives have not been investigated yet as a photosensitisers for PDT of atherosclerotic arteries or restenotic vessels, although this issue potentially may be interesting and clinically relevant.

A whole series of photosensitisers including fullerene Ñ60 and its derivatives is able to inactivate various types of viruses and, particularly, human immunodeficiency virus (HIV) [31]. The exact mechanism(s) of photodynamic virus inactivation remains obscure and may, conceivably, involve direct damaging effect of 1Î2, cleavage of viral DNA, and formation of new crosslinks in viral membrane and capsid proteins [32]. Taking into account viral theory of atherosclerosis suggesting the key etiological role of cytomegalo- and herpesviruses [33], it seems logical to assume that PDT of coronary vessels with fullerenes may theoretically be a useful tool in prevention of atherosclerosis. However, the detailed investigation of photodynamic antiviral effects of fullerenes is required to validate this concept.

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