In vitro studies on the cytoprotective properties of Carbon monoxide releasing molecules and N-acyl dopamine derivatives

Stamellou, Eleni

CHAPTER 3

N-acyl dopamine derivatives as lead compound for implementation in transplantation medicine.

Wedel Johannes, Pallavi Prama, Stamellou Eleni, Yard Benito

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Abstract

Conjugates of fatty acids with ethanolamine, amino acids or monoamine neurotransmitters occur widely in nature giving rise to so-called endocannabinoids. Anandamide and 2-arachidonoyl glycerol are the best characterized endocannabinoids activating both cannabinoid receptors (CB1 and CB2) and transient receptor potential vanilloid type 1 (TRPV1) channels (anandamide) or activating cannabinoid receptors only (2-arachidonoyl glycerol). TRPV1 is also activated by vanilloids, such as capsaicin, and endogenous neurolipins, e.g. N-arachidonoyl dopamine (NADA) and N-oleoyl dopamine (OLDA). Because donor dopamine treatment has shown to improve transplantation outcome in renal and heart recipients, this review will mainly focus on the biological activities of N-acyl dopamine derivatives (NADD) as potential non-hemodynamic alternative for implementation in transplantation medicine. Hence the influence of NADD on transplantation relevant entities, i.e. cold inflicted injury, cytoprotection, I/R-injury, immune-modulation and inflammation will be summarized. The cytoprotective properties of endogenous endocannabinoids in this context will be briefly touched upon.

Brain death

Donor organ shortage is the major bottle neck in contemporary organ transplantation and warrants new strategies to increase the donor pool, to diminish the number of organ allografts that are not suitable for transplantation, to improve post-transplant survival and thus to reduce the need for re-transplantation. While it is generally considered that the quality of organ allografts obtained from living donors is superior to that of allografts procured from post-mortem donors [138], the latter constitutes the largest part of the donor organ pool. The inferior quality of post-mortem donor allografts is a consequence of various deleterious events which occur after the onset of brain death. Brain death is characterized by a massive catecholamine release, initially leading to an increased blood pressure and subsequently to a sharp decline, frequently followed by a hemodynamic collapse [139]. In addition, BD is accompanied by reduced levels of cortisol, insulin, thyroid and pituitary hormones [140], which may have both a hemodynamic and metabolic impact on donor organs. Moreover, BD is considered to be an inflammatory condition [141], albeit that the precise mechanism that leads to inflammation in end-organs is still being discussed. Early and adequate donor management is of utmost importance not only for maintaining donor organ quality but also for increasing the number of retrievable organs from potential donors [142]. Thus many transplantation centers and critical care societies have developed standardized donor management protocols, focusing on hemodynamic and hormonal resuscitation [42, 143]

For many decades low-dose dopamine (DA) has been applied for prevention and treatment of acute kidney injury (AKI) in critically ill patients [144, 145]. However, several meta-analyses and prospective studies have concluded that DA treatment neither prevents nor ameliorates AKI in these patients [146-149]. This is in sharp contrast to the retro- and prospective studies performed by Schnuelle *et al.* which clearly indicate that donor dopamine treatment improves transplantation outcome in kidney and heart allograft recipients [45, 150]. The mechanism by which this occurs remains to be determined. Nonetheless, the protective effect cannot be fully explained by improved hemodynamics, as the mean blood pressure in the DA treated arm was not significantly different from the untreated arm [45]. Experimental brain dead models demonstrate that hemodynamic stabilization alone is not sufficient to reduce brain death mediated inflammation in renal allografts [151].In line with this, Spindler et al recently demonstrated that N-octanoyl dopamine (NOD) treatment of brain dead donor rats improves

renal allograft function in recipient rats, despite the fact that it does not affect blood pressure in the brain dead donor rat [152]

Cold inflicted injury

Experimental *in vitro* and *in vivo* studies have delineated numerous potential mechanisms that collectively explain why dopamine may afford its salutary effect on transplantation outcome [153-155]. However, it remains to be elucidated which of these effects are responsible for the clinical findings on donor dopamine treatment as described by Schnuelle et al. [45]. Amongst the beneficial mechanisms, the finding that catecholamines in general can protect endothelial cells against cold inflicted injury [156] is highly intriguing and opens the possibility for novel therapeutic modalities to prevent or limit organ damage during static cold storage [45, 150, 157-159]. The mechanism by which catecholamines protect against organ damage during hypothermic preservation is not completely understood, albeit that involvement of the H_2S pathway [160], the HO-1 pathway [161] and redox activity [156] have been postulated. With respect to the latter, Lösel *et al.* have demonstrated that the cryoprotective properties of catecholamine are mediated via the redox active catechol structure in conjunction with a minimal degree of hydrophobicity [162]. Importantly synthetic NADD, which also carry a catechol structure, are by far more protective as compared to dopamine as a consequence of their increased hydrophobicity. Furthermore octylamide derivatives of all possible dihydroxy benzoic acids revealed that only the reducing structures were protective while the non-reducing were ineffective despite comparable hydrophobicity.

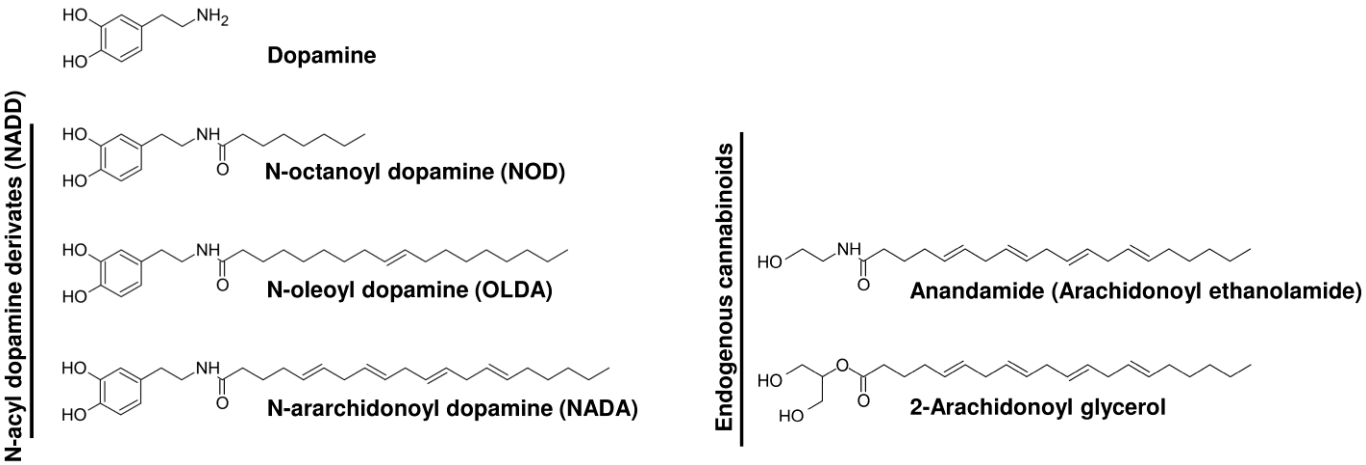

Figure 1: *Structure of dopamine, NADD and endocannabinoids*.

It thus seems that catecholamines afford cryoprotection due to their reducing properties; their efficacy is strongly influenced by their relative hydrophobicity.

Inasmuch as the catechol structure plays a pivotal role in cryoprotection independent of receptor engagement, this offers the possibility of designing compounds that are devoid of hemodynamic action and yet retain their cryoprotective properties. This might be of particular importance since approximately 12% of brain dead donors that are treated with low-dose dopamine may develop tachycardia or hypertension [45]. Also other dopamine-related side effects, *e.g.* depression of the respiratory drive or cardiac arrhythmias seem to be receptor-mediated [163, 164]. As demonstrated by Kohli *et al.* N-substituted dopamine derivatives lack affinity to dopamine receptors and only possess a weak beta agonistic activity [165]. Hence, NADD may display protective effects in the setting of donor treatment at much lower concentrations compared to dopamine, while dopamine-like side effects would occur at much higher concentrations as required for their protective effect. However, no clinical data on NADD are yet available, which impedes drawing firm conclusions on dopamine-related side effects or safety of NADD in humans. .

Nakao *et al.* have postulated that cytochrome P450 heme proteins are degraded during hypothermic organ preservation. This causes a detrimental increased level of intracellular free heme which subsequently leads to oxidative injury [39]. In addition to their reducing properties, catechol structures also have the propensity to coordinate with iron. Hence, it is at present unclear if the relevance of the catechol structure in preventing cold inflicted injury resides in its capacity to scavenge reactive oxygen intermediates or in preventing the formation of these intermediates via coordination with iron in the heme moiety.

Cytoprotective properties

The cytoprotective properties of endogenous NADD have been studied mainly in relation to brain function and neuroprotection, including positive effects on hypoxic–ischemic injury or brain inflammatory processes. Their protective effect is mainly attributed to the long-chain polyunsaturated fatty acids. Apart from the fact that dopamine fatty acid conjugates can act as cannabinoid receptor ligand [166] and as TRPV1 agonist [167], Shashoua *et al*. reported that some of these conjugates also act as carrier to increase brain dopamine content [168]. In line

with the tendency of docosahexaenoic acid (DHA) to accumulate in brain tissue [169], the DHA dopamine conjugate is most active in increasing dopamine uptake by the brain. Bobrov *et al*. showed that DHA-dopamine exhibited antioxidant activity and produced a dose-dependent protective effect on cultured granular cells from rat cerebellum under conditions of oxidative stress. It also decelerated the development of Parkinson's disease-like symptoms in a MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model [170]. The antioxidant properties of DHA-dopamine is most likely attributed to the catechol structure as it is also present in synthetic NADD with short saturated fatty acids, *e.g.* N-octanoyl dopamine (NOD) and N-pivanoyl dopamine.

Protection against I/R-injury

Even though our understanding in the pathophysiology of I/R-injury has improved to a large extent in the past decades, there is still a need for novel compounds that minimize the extent of tissue damage. Ischemic preconditioning (IPC) is a protective procedure accomplished by exposing the organ to a minor stress, which by itself does not cause noticeable harm. The benefit of IPC was first demonstrated by Murry *et al*. in dogs [171]; its protective potential on reperfusion injury is now widely accepted. When the heart was subjected, to short ischemic episodes separated by short perfusion periods the myocardium was more tolerant to subsequent prolonged ischemia. Although IPC is difficult to implement as a clinical strategy, identifying effective molecules in IPC can lead to new therapeutic treatment modalities. Yet, the underlying mechanisms of IPC has been equivocally discussed [172-174].

Proteasomal iron-protein degradation has been suggested by Bulvik *et al.* as important mechanism of IPC. In this scenario it is postulated that expeditious cytosolic iron release alters iron homeostasis which subsequently protects the myocardium during I/R [172]. In contrast Wu *et al.* proposed that the benefit of IPC was mediated via suppressing excessive endoplasmic reticulum stress thereby diminishing C/EBP homologous protein (CHOP)-dependent apoptosis [173]. Also suppression of cardiac progenitor cell apoptosis has been suggested [174]. More recently Lu *et al.* suggested a role of TRPV1 in IPC [175]. Their data suggest that IPC upregulates arachidonate 12-lipoxygenase (ALOX12) and consequently increases the production of the endovanilloid 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE), which in turn activates TRPV1. It is believed that TRPV1 functions as polymodal sensor to detect micro-environmental changes in tissues, *e.g.* low pH, high temperature, or noxious stimuli [176, 177].

These changes are likely present in ischemic tissue. Experimental evidence in TRPV1$^{-/-}$ mice have indicated that tissue damage as a consequence of AKI is much more severe as compared to that of wild type mice, suggesting that TRPV1 activation may function as feedback loop to limit ischemic insults. The finding that NOD activates TRPV1 may explain its renoprotective properties in the setting of ischemia-induced AKI [178], yet the causality of this observation and its translation to clinical application warrants further supportive evidence.

Immune modulation and inflammation

The main pharmacological functions of the endocannabinoid system include neuromodulation, controlling motor functions, cognition, emotional responses, homeostasis and motivation. In the periphery, this system is also an important modulator of immunity [179].

Several studies have unambiguously demonstrated that endocannabinoids modulate proliferation and apoptosis of T and B lymphocytes, macrophage-mediated killing, cytokine production, immune cell activation by inflammatory stimuli, chemotaxis and inflammatory cell migration [180, 181]. Most if not all of these effects have been reported to be primarily mediated via CB2 receptors causing inhibition of the cAMP/protein kinase A (PKA) pathway.

NADD may modulate the immune system in a similar fashion. Yet there are a number of biological activities described for NADD related to immune modulation that are neither mediated via CB receptors nor via TRPV1. Initially NADD were described as potent inhibitors of 5-lipoxygenase (5-LO) [182]. This enzyme catalyzes two steps in the biosynthesis of leukotrienes (LT), a group of lipid mediators of inflammation derived from arachidonic acid (AA). LT antagonists are used in treatment of asthma; more recently a potential role in neointimal thickening and atherosclerosis has raised considerable interest [183-186]. Cyclooxygenase-2 (COX-2) is an enzyme that plays a key role in inflammatory processes. Classically, this enzyme is upregulated in inflammatory situations and is responsible for the generation of prostaglandins (PG) from AA. One lesser-known property of COX-2 is its ability to metabolize the endocannabinoids, N-arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG), generating PG-glycerol and PG-ethanolamides [187]. Although the formation of these COX-2-derived metabolites of the endocannabinoids have been known for a while, their biological effects remain to be fully elucidated. Recently, Alhouayek *et al*. showed that 2-AG through its oxygenation by COX-2 gives rise to the anti-inflammatory prostaglandin D_2-glycerol ester (PGD_2-G) [188].

Interestingly, Navarrete *et al.* found that NADA activates a redox-sensitive p38 MAPK pathway that stabilizes COX-2 mRNA resulting in the accumulation of the COX-2 protein [189]. Moreover, they demonstrated that NADA inhibits the expression of microsomal prostaglandin E_2 synthase 1 (mPGES-1) and thus the production of the inflammatory mediator PGE_2. This was paralleled by the induction of lipocalin-type prostaglandin D synthase (L-PGDS) and increased production of PGD_2. Therefore even though COX-2 is involved in inflammation in conjunction with NADD, in particular NADA, it may redirect PG synthesis towards formation of PGD_2 [190]. The finding of Patel *et al.* that selective inhibitors of COX-2 may worsen renal dysfunction and injury in conditions associated with renal ischemia support this view [191].

Because of their redox active catechol structure NADD have the propensity to inhibit the activation of redox dependent proinflammatory transcription factors, *e.g.* NF-κB, AP-1 and NFAT [192, 193], and therefore they largely inhibit the expression of inflammatory mediators produced by endothelial cells [194] and proliferation of T cells [192, 193, 195].

Implementation of NADD in transplantation medicine

While the list of compounds that show beneficial effects at the pre-clinical stage is steadily increasing, only a limited number of such compounds will find a clinical use. The lack of venture capital for entering clinical phase studies is amongst others why only a small percentage of promising compounds will proceed, let alone will obtain FDA approval.

The multitude of beneficial effects of NADD warrants careful considerations on the application mode, e.g. donor or recipient treatment, additive to preservation solutions, and their associated ethical hurdles. Particularly donor pre-treatment may raise ethical concerns as to whether written informed consent of the recipient is required. Waiving informed consent of the recipient in the randomized donor dopamine treatment trial was justified because it was 1) strictly observational in the recipient, 2) the intervention was limited to the deceased donor and 3) limited to a fully approved drug. Clearly, as FDA approval for the use of NADD in human does not exist, similar studies with the use of NADD are not possible. Although NADD may also have the propensity to protect allografts when used as additive to the organ preservation solution, there is no supportive evidence using whole organs, albeit that it has been reported for experimental models that addition of dopamine to the preservation solutions is protective to liver [52] and renal allografts [159]. Also the type of NADD for implementation in transplantation medicine requires careful considerations since some of the beneficial effects of NADD are meditated by the fatty acid tail and therefore not present in all NADD.

Concluding remarks

This review has summarized the potential protective properties of NADD on transplantation relevant entities. Based on their propensity to act as agonist of CB receptors and TRPV1 channels, to act as anti-oxidant and to inhibit inflammatory mediators including those derived from arachidonic acid, NADD may find clinical implementation in transplantation medicine as a mean of pre-conditioning to prevent brain death-induced inflammation and to prepare donor organs to cold ischemia. Yet it should be emphasized that most of the potential benefits of NADD mainly have been studied in vitro and only to a limited extent in transplantation relevant in vivo models. Nonetheless their potential as new expedient drugs should be further explored using relevant transplantation models. The composition of the NADD, i.e. the type of fatty acid

that is required for a specific in vivo biological effect, as well as the pharmacokinetic of these compounds should be implemented in future studies.