RESEARCH ON THE ADVERSE REACTIONS TO IODINATED CONTRAST AGENTS. THE ROLE OF 5-PHOSPHODIESTERASE INHIBITORS IN PREVENTING CONTRAST NEPHROPATHY

PhD SUPERVISOR:
UNIV. PROF. DR. CĂLINA CORNELIA-DANIELA

PhD STUDENT:
IORDACHE MIHAI-ANDREI

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# Table of Contents

1. INTRODUCTION .................................................................................................................. 1  
2. CURRENT KNOWLEDGE BASE ........................................................................................... 1  
   2.1 Pharmacokinetics ............................................................................................................. 1  
   2.2 Adverse reactions after administering iodinated contrast agents ............................... 1  
3. PERSONAL CONTRIBUTIONS ............................................................................................... 3  
   3.1 General aims .................................................................................................................... 3  
   3.2 Materials and methods .................................................................................................... 3  
      3.2.1. Cutaneous symptoms indicating hypersensitivity reactions caused by iodinated contrast agents in comparison to other contrast agents .............................................. 3  
      3.2.2. The effects of 5-Phosphodiesterase inhibitors (PDE5I) in iodinated contrast induced nephropathy in rats (CIN) ................................................................. 3  
3.3 RESULTS .......................................................................................................................... 4  
      3.3.1 The effects of 5-Phosphodiesterase inhibitors (PDE5I) in iodinated contrast induced nephropathy in rats .................................................................................... 5  
      3.3.2 Oxidative stress evaluation ....................................................................................... 5  
      3.3.3 Histopathological evaluation .................................................................................... 6  
4. Discussions ......................................................................................................................... 6  
   4.1. Cutaneous symptoms indicating hypersensitivity reactions caused by iodinated contrast agents in comparison to other contrast agents ......................................................... 6  
   4.2 The effects of 5-Phosphodiesterase inhibitors (PDE5I) in iodinated contrast induced nephropathy in rats .............................................................................................. 6  
5. Conclusions ......................................................................................................................... 8  
6. Selected bibliography .......................................................................................................... 9

**Keywords:** Iodinated contrast agents, side effects, hypersensitivity, skin lesions, contrast induced nephropathy, phosphodiesterase 5 inhibitors, oxidative stress.
1. INTRODUCTION

Adverse reactions to contrast agents can be classified into non-renal and renal adverse reactions (1). Non-renal adverse reactions can be further divided into immediate (2) and delayed (3) adverse reactions. One of the aims of this doctoral thesis is to create a systematical analysis of the existing body of research from the past 10 years in order to identify risk factors, types of non-renal adverse reactions as well as their prevalence, thus facilitating the identification of patients who are more likely to encounter these adverse reactions.

Contrast induced nephropathy (CIN) appears rapidly after the administration of iodinated contrast agents and it triggers an increase in the serum creatinine, which can be determined 48 hours after administration (1, 4). The second aim of this doctoral thesis is to bring a pioneer investigation of the comparable therapeutic potential of Sildenafil and Tadalafil in preventing CIN. In order to verify this hypothesis, we have used animal models with CIN, and compared the efficacy of PDE5 inhibitors with that of N-acetyl cysteine (NAC), used in the current practice as a preventive treatment.

2. CURRENT KNOWLEDGE BASE

2.1 Pharmacokinetics
The pharmacokinetics of contrast agents can be visualized through a bicompartamental model made up of a central compartment, blood, and a second compartment, the extracellular space.

Iodinated contrast agents are not metabolized; they are eliminated without modification through filtration by the renal glomerulus. In patients with a normal renal function, their elimination is effectively handled up to 80% in the first 4 hours, and up to 98% in the first 24 hours after administration. Renal insufficiency constitutes a direct impact on the rate of excretion.

2.2 Adverse reactions after administering iodinated contrast agents
There are three major categories of adverse reactions: acute reactions, delayed non-renal reactions and renal reactions, or contrast induced nephropathy (CIN).

Non-renal reactions
Acute reactions usually appear within an hour of administering iodinated contrast agents and can vary in terms of clinical pictures and severity. They can be further categorized into anaphylactoid or idiosyncratic reactions (dose dependent) and non-anaphylactoid or chemotoxic reactions (dose independent) (2, 3).

Delayed adverse reactions comprise of clinical symptoms that appear after at least 30-60 minutes and up to a week after administering iodinated contrast agents (4).
Renal symptoms or contrast induced nephropathy (CIN)

Administering iodinated contrast agents can provoke renal symptoms very similar to acute renal injury in certain patients (5). Despite the lack of standard criteria for CIN, a diagnosis can be made if in 48-72 hours from administering the iodinated contrast agent, one of the following modifications appear:

1. serum creatinine grows with 0.3 mg / dL (26 µmol/l)
2. a 50% relative growth of serum creatinine in comparison with the base level
3. reduced urine production to less than 0.5 ml / kg / hour for at least 6 hours (5).

The exact physiopathology of CIN is still unknown, but it is believed to be caused by a combination of multiple mechanisms including tubular toxicity, endothelial toxicity and renal ischemia induced by hemodynamic imbalance (4, 5).

Cytotoxicity is determined by the iodine in the contrast agents, which has a direct impact on the tubular epithelial cells by triggering their vacuolation and the osmotic nephrosis of endothelial cells (6).

Moreover, iodinated contrast agents have a high oxidative capacity, stimulating the release of reactive oxygen species (ROS). ROS, like superoxide (O2\(^-\)), hydrogen peroxide (H\(_2\)O\(_2\)) and hydroxyl radicals (OH\(^-\)), are actively involved in the inflammatory response.

Impairment of renal hemodynamics. ROS determines the growth of endothelin synthesis, angiotensin II, angiotensin, as well as the accumulation of peroxynitrite, which reduces the bioavailability of nitric oxide, causing a vasoconstriction of vasa recta and peritubular and glomerular capillaries by means of endothelial cell dysfunction (7).
3. PERSONAL CONTRIBUTIONS

3.1 General aims

The hypotheses of this doctoral thesis started from the analysis of the possible hypersensitivity and nephrotoxicity reactions of iodinated contrast agents and their connection with the risk factors.

In the first part of the doctoral thesis, we have conducted a systematic analysis of studies published in the last ten years on the incidence of immediate and delayed hypersensitivity reactions to iodinated contrast agents with cutaneous manifestations. The aim of this analysis was to identify the types of skin manifestations characteristic of these types of reactions and to analyze the risk factors and comorbidities that may influence their appearance.

Phosphodiesterase 5 inhibitors (PDE5I) produce a vasodilatation mediated by nitric oxide (NO), having positive effects on the renal hemodynamic (19, 20). Starting with this hypothesis, the second part of this paper attempts to provide an analysis of the protective effects of Sildenafil and Tadalafil (19, 21), two PDE5I, in CIN in a rat model. Another goal was to identify the role of oxidative stress in CIN, and determine if Sildenafil and Tadalafil are related to its modulation, as well as to evaluate the histopathological modifications.

3.2 Materials and methods

3.2.1. Cutaneous symptoms indicating hypersensitivity reactions caused by iodinated contrast agents in comparison to other contrast agents

In the first study of the doctoral thesis, the main method has been systematic analysis. The initial evaluation of studies published in the English language between January 2008 and January 2018 has identified 929 articles. After excluding the studies rendered inconsistent with the inclusion criteria, 20 articles were selected for this analysis.

3.2.2. The effects of 5-Phosphodiesterase inhibitors (PDE5I) in iodinated contrast induced nephropathy in rats (CIN)

The experimental design of in vivo study

The 30 rats were divided into 5 groups as follows:
- CTRL Group (control) (n = 6; PBS was administered once a day by gavage for 7 days without triggering CIN)
- CIN group (n = 6; PBS was administered once a day by gavage for 7 days before inducing CIN)
- CIN + SIL group (Sildenafil) (n = 6; Sildenafil 10 mg / kg c / day was administered by gavage for 7 days before inducing CIN). The dose of Sildenafil was determined based on the doses previously reported in rats (8).
- CIN + TAD group (Tadalafil) \( (n = 6) \); Tadalafil 5 mg / kg/day was administered by gavage for 7 days before inducing CIN. The dose of Tadalafil was determined based on the doses previously reported in rats (9).

- CIN + NAC group \( (n = 6) \); NAC 100 mg / kg/day was administered by gavage for 7 days before inducing CIN; positive control for the protection against CIN (10).

CIN was induced based on existing protocols (11) as follows:

For biochemical analysis, blood was drawn from the vein in the tail. The blood draw was made before inducing CIN, as well as 24 hours after inducing CIN. In order to evaluate the oxidative stress markers, blood tests were made 24 hours after inducing CIN. For the histological evaluation of tissue, sections of approximately 25 µm were cut using a microtome and colored with hematoxylin/eosin.

### 3.3 RESULTS

#### 3.3.1 Cutaneous symptoms indicating hypersensitivity reactions caused by iodinated contrast agents in comparison to other contrast agents

Studies published in the last 10 years that assessed the incidence of immediate adverse reactions to iodinated contrast agents have identified a prevalence of immediate hypersensitivity reactions between 0.16% and 2.24%, with skin manifestations accounting for between 33.33% and 87.7%. The most common skin manifestations were urticaria, rash, pruritus, edema, erythema and angioneurotic edema.

**Incidence of immediate skin adverse reactions of non-iodinated contrast agents.** The incidence of immediate hypersensitivity reactions to Gadolinium was very low, 0.01% - 0.3%. Urticaria, rash, pruritus and limited facial edema were the most common clinical manifestations. In the investigated studies, sulfur hexafloride did not show side effects, and Fluorescein had an incidence of 1.4%, of which 28.12% were skin effects.
Delayed hypersensitivity reactions to iodinated agents have also been reported in studies published in the last ten years that investigated the safety of iodinated contrast substances (ICSs). A prevalence between 0.42% and 14.3% was observed, of which the incidence of skin manifestations was between 43.05% and 100%. The most common cutaneous manifestation was angioedema (between 11.1% and 43.7%), pruritus (18.7-55.6%), maculopapular rash (33.3-37.5%).

Delayed skin reactions of non-iodinated contrast agents have been investigated in only 3 studies published in the last 10 years. Two studies evaluated the safety of sulfur hexafluoride in the pediatric population and did not report any delayed adverse events, and in the case of Gadolinium-based agents, an incidence of 0.05% was reported.

We also identified several risk factors associated with iodinated contrast agents. Females were associated with an increased risk of immediate allergic reactions to ICS between 51.44% and 65.95%. Other identified risk factors included history of previous reactions to ICS (1.2-11.6%), atopy (14.3%), asthma (2.1% - 12.7%), drug allergy (3.6%) - 25% and allergic rhinitis (1.5% - 4%). Immediate allergic reactions were also associated with concomitant treatment with beta-blockers (7.9%) or angiotensin converting enzyme inhibitors (ACE inhibitors) (13.2%). In the case of non-iodinated substances, females were a risk factor when using Gadolinium-based products (65.2% - 81.25%), along with previous adverse reactions (7.31 - 8.5%), asthma (2.9% - 11%), hypersensitivity to drugs (2%).

3.3.2 The effects of 5-Phosphodiesterase inhibitors (PDE5I) in iodinated contrast induced nephropathy in rats

The validity of the model was demonstrated, as all the animals in the CIN group presented a sCr growth of over 25% compared to the baseline value 24 hours after administering ICS (12). The variability coefficient (%) VC of the serum creatinine 24 hours after inducing CIN had increased significantly from the baseline level (with 104%) in the CIN group, in comparison to the control group (CTRL).

The variation of the serum creatinine measured 24 hours after inducing CIN was significantly reduced in all the treatment groups (SIL, TAD, NAC) in comparison to the CIN group (p < 0.050), but there were no variations among these groups. The sCr and the urea levels had grown significantly 24 hours later in the CIN group, in comparison to the control group. In addition, a notable decrease was detected in the CIN + SIL, CIN + TAD and CIN + NAC groups in comparison to the CIN group, but not in comparison to the CTRL group.

Oxidative stress evaluation

Oxidative stress was evaluated using reduced glutathione (GSH), catalase (CAT), the reactive components of thiobarbituric acid (TBARS), protein carbonite (PROTC) and the total antioxidant capacity (TAC).

The oxidative stress markers, TAC, GSH and CAT, showed a meaningful decrease in comparison to the CTRL group, and an increase in the CIN+SIL, CIN+TAD and CIN+NAC
groups. Meanwhile, the TBARS and PROTC levels grew markedly in comparison to the CTRL group and decreased in CIN + SIL, CIN + TAD and CIN + NAC groups.

**Histopathological evaluation**

The control group (CTRL) presented a regular aspect of the kidneys. The CIN group showed hydrotropic modifications in the renal tubules (proximal tubules, twisted distal and loop of Henle), a growth of the Bowman space, lobulated glomera and the alteration of the macula densa of the twisted distal tubules. The CIN + SIL / CIN + TAD groups did not show histological changes. The CIN + NAC group exhibited similar histopathological changes, but less severe in comparison to the CIN group.

4. **Discussions**

4.1. **Cutaneous symptoms indicating hypersensitivity reactions caused by iodinated contrast agents in comparison to other contrast agents**

In our systematic research, the frequency of adverse reactions in iodinated contrast agents (ICA) was registered between 0.12% and 1.15%, according to the bracket showcased in the studies (13, 14). Among the reactions of immediate hyper sensibility, cutaneous symptoms have constituted between 33.33% and 87.7%, with most studies reporting percentages of over 50%. Non-iodinated contrast agents have a safer profile compared to ICS, with the incidence of immediate adverse reactions being very low in gadolinium-based contrast agents and other agents used for enhanced contrast ultrasound (CEUS). In this case, the lower rate of adverse reactions is probably due to the limited data available in the literature.

**Immediate adverse reactions.** The most common adverse reactions were hives, rash and pruritus. Immediate adverse reactions were also identified, and anaphylactic symptoms were often encountered. The main mechanism responsible for this is the hyper sensibility type I mechanism. Iodinated contrast agents have a safer profile in comparison to ICA.

**Delayed adverse reactions.** In the studies used, the frequency of delayed reactions in ICA was between 0.03% and 10.1% of the cases. The physio-pathological mechanism of these delayed reactions is thought to be mediated by T cells.

**Risk factors.** All of the following attributes have been identified as risk factors: atopic terrain, previous reactions to ICA, being under 50, being female, and having a history of cardiac disease and a concomitant treatment with drugs such as beta-blockers or aldosterone converting enzyme inhibitors (ACEI) (15, 16). Asthma has been associated with severe reactions.

4.2 **The effects of 5-Phosphodiesterase inhibitors (PDE5I) in iodinated contrast induced nephropathy in rats**

In this thesis, we have induced nephropathy using contrast agents in a rat animal model according to existing protocols, by using indomethacin as inhibitor for the prostaglandin synthesis and L-NAME as inhibitor of NO synthesis (11, 17). Iopromide, an agent frequently used in medical practice, was used. Two phosphodiesterase 5 inhibitors (PDE5I) were used—
Sildenafil (10 mg/kg/day) and Tadalafil (5 mg/kg/day) for 7 days before administering the contrast agent. Sildenafil and Tadalafil act by inhibiting phosphodiesterase 5 (PDE5), tasked with tying and hydrolyzing cyclic guanosine monophosphate (cGMP), a molecule that produces NO. Therefore, by inhibiting the degradation of cGMP, the NO action is prolonged, stimulating the vasodilation (18).

In the CIN group, all animals have developed nephropathy, defined by the growth of creatinine by 25% in contrast with base level values, which has demonstrated the validity of the model. No consequential differences have been detected among the serum levels of creatinine, urea, sodium, potassium and chlorine in the groups where a pre-treatment consisting of Sildenafil, Tadalafil and N-acetyl cysteine was administered. Pre-treatment with Sildenafil and Tadalafil has proved meaningful in limiting the growth of creatinine levels in comparison to the CIN group, and is comparable to those of the NAC group.

**Monitoring oxidative stress**

Oxidative stress was evaluated using reduced GSH, as well as CAT, TBARS, TAC and PROTC. GSH is the main non-enzymatic antioxidant involved in purifying free radicals. CAT is an antioxidant enzyme involved in protecting cells by the deterioration caused by peroxide (19). TBARS represent the markers of lipid peroxidation, and their level is augmented in oxidative stress (20). PROTC is a marker of the oxidative deterioration of proteins, and its level grows in oxidative stress. TAC is a marker of the antioxidant capacity of the plasma (21).

This paper demonstrates that in the CIN group, the TAC, GSH and CAT levels have significantly decreased, while the TBARS and PROTC levels have significantly increased when compared to the control group, which is also in accordance with previous studies (22-24).

**Histopathological evaluation**

Histopathological evaluation of the kidneys has highlighted regular kidneys in the control group, hydropic changes of the renal tubules (proximal tubules, twisted distal and loop of Henle), augmented Bowman space, lobulated glomera and alteration of the macula densa region of twisted distal tubules in the CIN group. The CIN + SIL / CIN + TAD have shown a normal renal histology. The CIN + NAC group has presented similar histopathological changes, but less severe that in the CIN group.
5. Conclusions

Contrast agents are used today for a wide range of imaging investigations, and their use is not entirely safe, as they are responsible for the occurrence of immediate or delayed side effects. Iodinated contrast agents have a higher incidence of side effects compared to new agents such as gadolinium-based contrast agents or others used for contrast-enhanced ultrasound. Skin manifestations are the most common manifestations of all types of allergic reactions, and an accurate diagnosis can be very useful in practice. Several risk factors have been associated with the occurrence of immediate hypersensitivity reactions to contrast agents. Proper medical history and prophylactic treatment can limit the incidence and severity of side effects and also prevent life-threatening reactions.

In this doctoral thesis, I have also studied the effects of 5-Phosphodiesterase inhibitors, Sildenafil and Tadalafil, on a rat model with contrast agent induced nephropathy. I have monitored the biochemical markers, the markers of oxidative stress and the histopathological modifications, and I have compared the results obtained in each experimental group.

From the point of view of histopathological modifications, CIN induces structural renal lesions in rats, as well as hydropic modifications of the renal tubules (proximal tubules, twisted distal and loop of Henle), augmented Bowman space, lobulated glomera and alteration of the macula densa region of twisted distal tubules. Pre-treatment with Sildenafil and Tadalafil improves the structural renal lesions triggered by CIN, and the protective potential of these drug agents is superior to that of NAC.

Inducing CIN in a rat model leads to disorders of the redox status, which in turn leads to a decrease of TAC, GSH and CAT levels, and a growth of the TBARS and PROTC levels. Pre-treatment with Sildenafil and Tadalafil reduces the frequency of CIN by modulating the pro-oxidant/ antioxidant equilibrium. Their effects are superior to those of NAC, even if they have not yet reached the statistical significance, thus making them good candidates for future studies that intend to demonstrate their positive effects in combating CIN. I have confirmed that oxidative stress is one of the molecular mechanisms involved in the CIN pathogenesis, and determining that oxidative stress markers such as TAC, GSH, CAT, TBARS and PROTC can be used as biomarkers for the monitoring of CIN.
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