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PROTOCOL QUERCETIN (Q-CABG)

The Anti-inflammatory and Senolytic Effect of Quercetin During Cardiac Surgery

Sponsors or Funding Organizations

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1. Introduction and Definition of the Research Problem

Quercetin is a naturally occurring flavonoid with anti-inflammatory and antioxidant effects. Numerous studies have investigated the effects of quercetin in animals. However, few studies have addressed the medical use of quercetin in humans.

The purpose of this study is to evaluate the effect of quercetin on the inflammatory response following cardiac surgery, as well as its senolytic effect on endothelial cells, primarily in patients with unstable coronary artery disease, such as those undergoing myocardial revascularization after a myocardial infarction or with clinical unstable angina.

2. Relevant Literature Review

In a recent clinical study involving 47 patients, we observed increased blood levels of high-sensitivity reactive C protein (hs-CRP) and angiopoietin-like 2 (ANGPTL2)—a pro-inflammatory adipokine—24 hours after cardiac surgery (1). After surgery, ANGPTL2 levels increased in a group of older patients with higher blood pressure, while levels decreased in younger patients with lower blood pressure (1) (Table 1). hs-CRP levels remained relatively stable and did not mirror the ANGPTL2 secretion profile; however, hs-CRP levels did reflect acute inflammation partly caused by extracorporeal circulation and the surgical procedure.

In a preliminary analysis assessing endothelial dysfunction, single-cell mRNA sequencing of the internal mammary artery endothelium identified nine significantly overexpressed genes in patients with unstable coronary artery disease (Table 2). These genes were associated with targets of senolytic agents—such as quercetin (which eliminates senescent cells)—and with cardiovascular diseases, reflecting the severity of endothelial dysfunction and atherosclerotic involvement.

Effect of Quercetin

Quercetin is a flavonoid found in plants, fruits, vegetables, and red wine. It is thus a natural compound, regularly consumed through dietary plant products. Dietary intake varies considerably but is estimated between 10 and 100 mg per day (2). It has well-demonstrated anti-inflammatory and antioxidant effects in both animals and humans (3–8). Quercetin slows the progression of atherosclerosis in rabbits (4). It also reduces the activity of inflammatory mediators such as cyclooxygenase, myeloperoxidase, NOS, and serum CRP levels in animals with aortic aneurysms (7), and has antihypertensive effects through renal sodium regulation mechanisms (9). It has also been proposed as a potential antidiabetic agent based on animal studies (10).

However, there are still few human studies involving quercetin supplementation for medical purposes, particularly for cardiovascular disease prevention (11).

Surprisingly, quercetin has been extensively studied in sports medicine to evaluate its impact on physical performance in athletes and soldiers (2,12,13). A dose of 1 g per day for three weeks did not reduce inflammation in young subjects but improved muscle performance (14); it was the first study to show that quercetin improves muscle function and attenuates immune alterations

after exercise. These results were confirmed by McAnulty's team (13). The absence of an anti-inflammatory effect may be due to the fact that participants were young and physically active. Indeed, a daily dose of 500 mg (15) and 730 mg (16) for 10 and 4 weeks respectively reduced blood pressure and inflammation (IL-6, TNF-alpha) in women with type 2 diabetes and reduced

Tableau 1. Comparaison entre les paramètres préopératoires entre les deux groupes de patients avec un ΔANGPTL2 positif (groupe *INCREASED*) ou négatif (groupe *DECREASED*) (1).

Parameter	Negative Δ (ANGPTL2 decreased; n = 21)	Positive Δ (ANGPTL2 increased; n = 26)	*P* value
Age, y	63 ± 2	71 ± 1	**.002**
BMI	28.2 ± 4.4	29.7 ± 5.1	.405
Obesity	8 (38)	14 (54)	.282
Women	4 (19)	6 (23)	.737
Type 2 diabetes	7 (33)	11 (42)	.529
Dyslipidemia	16 (76)	22 (84)	.466
Hypertension	14 (67)	21 (81)	.270
SBP, mm Hg	124 ± 5 (19)	136 ± 3 (23)	**.038**
DBP, mm Hg	71 ± 4 (19)	74 ± 2 (23)	.866
HR, beats per minute	79 ± 3	73 ± 2	.078
Active smoker	3 (14)	4 (15)	.916
Ex-smokers	6 (29)	7 (27)	.900
COPD	1 (5)	4 (15)	.240
Family history	5 (24)	9 (35)	.421
Chronic renal dysfunction	1 (5)	3 (12)	.408
Previous MI	5 (24)	5 (19)	.703
Preoperative AF	5 (24)	7 (27)	.800
At least 6 risk factors for CVD	11 (52)	17 (65)	.108

blood pressure in patients with stage 1 hypertension (140–159 mmHg systolic/90–99 mmHg diastolic), but not in pre-hypertensive individuals. Thus, the anti-inflammatory effects of quercetin may only be apparent in patients with cardiovascular risk factors. It is therefore

relevant to test a 1000 mg/day dose over a short period to assess its anti-inflammatory effects during cardiac surgery in a population already considered "inflamed."
Bioavailability varies depending on the oral formulation used in humans (5,17). Notably, no adverse effects have been reported from oral administration of quercetin at doses ≤1200 mg/day for up to 12 weeks (2,17).

Table 2. Main Genes Differentially Expressed in Endothelial Cells of the Mammary Artery from a Patient with Stable Coronary Artery Disease and a Patient with Unstable Coronary Artery Disease.

Gene	Stable CAD Patient	Unstable CAD Patient	P-value *(Benjamini-Hochberg corrected)*	Associated Condition
TGFB2	0.0%	41.7%	0.00061	Atrial fibrillation
SQLE	0.0%	41.7%	0.00061	Statins
RPPH1	2.9%	58.3%	0.00090	Quercetin
ARL14EP	7.1%	66.7%	0.00339	Systolic blood pressure
RPL17	25.7%	100.0%	0.00914	Myocardial ischemia
IGFBP7	17.1%	83.3%	0.01556	Ruptured aneurysm
TMEM8B	0.0%	33.3%	0.01699	Valvular heart disease
ZNF675	0.0%	33.3%	0.01699	Congestive heart failure
SELL	8.6%	66.7%	0.04848	Vaso-occlusive crisis

Percentages represent the number of endothelial cells expressing each gene per patient.

3. Objectives and Evaluation Criteria

3.1 Primary Objective

The primary objective of this study is to evaluate the effect of quercetin (500 mg BID; 1 g/day) on the inflammatory response four days after cardiac revascularization surgery in patients with unstable coronary artery disease—such as those undergoing myocardial revascularization following a myocardial infarction or experiencing clinical unstable angina.

3.2 Secondary Objective

The secondary objective is to assess the senolytic effect of quercetin (500 mg BID) on endothelial cells following revascularization surgery.

3.3 Exploratory Objective

The exploratory objective is to study the effect of quercetin (500 mg BID) on the inflammatory response at additional time points following revascularization surgery—specifically, 24 hours post-surgery and at hospital discharge (or up to a maximum of seven days post-surgery).

3.4 Primary Evaluation Criterion

The primary evaluation criterion is the change in blood levels of hs-CRP between baseline (t-1) and four days after surgery (t2).

3.5 Secondary Evaluation Criteria

Secondary evaluation criteria include:

- Change in blood levels of ANGPTL2 between baseline (t-1) and four days after surgery (t2);
- Endothelium-dependent relaxation function;
- Expression of arterial markers of inflammation and senescence, assessed by:
    - Ex vivo measurement of endothelial relaxation function induced by acetylcholine;
    - mRNA assays in isolated wall cells.

3.6 Tertiary / Exploratory Evaluation Criteria

Exploratory criteria include changes in hs-CRP and ANGPTL2 levels between baseline (t-1) and two other time points: 24 hours after surgery (t1) and at hospital discharge (or up to a maximum of seven days post-surgery) (t3).

Objectives – Evaluation Criteria – Rationale Table

Objectives	Evaluation Criteria	Justification
Primary	Change in hs-CRP levels between baseline and day 4 (t2)	hs-CRP and ANGPTL2 blood levels are expected to peak on day 4 post-surgery, based on Figures 1 and 2.
	Change in ANGPTL2 levels between baseline and day 4 (t2)	Secondary criterion for the same objective.

Objectives	Evaluation Criteria	Justification
Secondary	Endothelial relaxation function (acetylcholine, ex vivo); mRNA assays	The nine genes identified via mRNA sequencing were associated with senolytic targets including quercetin and cardiovascular disease, reflecting severity of endothelial dysfunction.
Exploratory	Change in hs-CRP and ANGPTL2 at t1 (24h) and t3 (hospital discharge)	These exploratory criteria are included to validate inflammatory markers at additional time points, as shown in Figures 1 and 2.

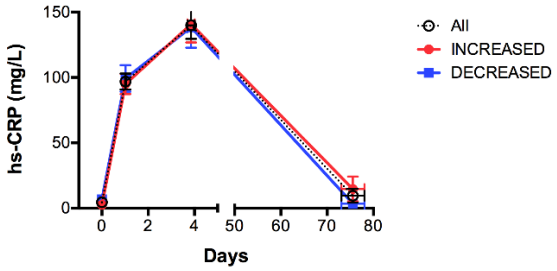

Figure 1. Mean plasma levels of hs-CRP post-surgery in all patients (*All*) and in the group where ANGPTL2 increased (*INCREASED*) or decreased (*DECREASED*) (see Figure 2). Data are means ± SEM of 47 patients. Two way ANOVA for repeated measures (Group effect: p=0.997; time: p<0.0001; Groupe x time: p=0.375) (1).

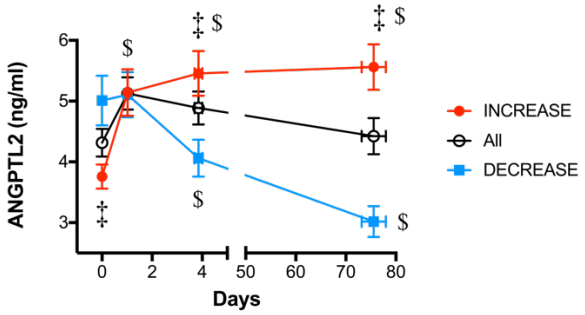

Figure 2. Circulating levels of ANGPTL2 decreased (group *DECREASED*, n=21) or increased (group *INCREASED*, n=26) at the end of the follow-up (76 days post-surgery). The opposite profils of change are presented. For comparaison, the average profil of ANGPTL2 evolution in plasma as a fonction of time post-surgery is shown (group *All*).

Data are means ± SEM of 47 patients. Two way ANOVA for repeated measures (interaction between Group and Time $p<0.0001$); ‡: $p<0.05$ *versus DECREASED*; $: $p<0.05$ *versus* basal (t=0), in each group of patients) (1).

4. Methodology

4.1 Study Design

This research is a randomized, placebo-controlled study. Patients undergoing myocardial revascularization surgery following a myocardial infarction or presenting with unstable angina will be approached to participate in the study. These are patients awaiting surgery in the coronary care unit. All patients will undergo coronary artery bypass grafting (CABG) using standard and recognized procedures.

Five milliliters of blood will be collected at four time points: preoperative (t-1), 24 hours post-op (t1), four days post-op (t2), and at hospital discharge (or up to a maximum of seven days post-op) (t3), to measure plasma levels of hs-CRP and ANGPTL2.

A segment of internal mammary artery discarded during bypass surgery will be retrieved for:

1. Measurement of ex vivo endothelial relaxation function induced by acetylcholine;
2. mRNA analysis in cells isolated from the arterial wall.

4.2 Description of the Population

As mentioned previously, patients undergoing myocardial revascularization surgery following a myocardial infarction or who present with unstable angina will be considered for study enrollment. These patients will be hospitalized in either the coronary care unit or the cardiac surgery unit, possibly transferred from a referring center.

4.2.1 Inclusion Criteria

- Aged 18 years or older;
- Able to give free and informed consent;
- Able to communicate in French or English;
- Hospitalized and awaiting cardiac revascularization surgery;
- Have had a myocardial infarction in the past 30 days or present with unstable angina preoperatively.

4.2.2 Exclusion Criteria

- Stable condition without myocardial infarction in the past 30 days;
- Undergoing concomitant cardiac surgery along with revascularization;

- History of infection in the past 30 days;
- Renal failure (GFR < 30);
- Liver impairment (AST, ALT, or bilirubin > 2× normal values);
- Known cirrhosis;
- History of breast cancer or other estrogen-dependent tumors;
- Intolerance to flavonoids, niacin, or ascorbic acid;
- Requiring a quinolone in the postoperative period;
- Inability to give informed consent;
- Inability to communicate in French or English.

4.2.3 Strategy for Recruiting and Retaining Study Participants

The study will be presented to patients hospitalized in the relevant units who meet the inclusion/exclusion criteria. The research team will present the study and provide the information and consent form to potential participants for thorough review. Any questions will be answered by the research staff.

One hundred (100) patients will be recruited and randomly assigned into the two study groups using a randomization table (quercetin 500 mg BID vs. placebo).

4.3 Study Plan and Conduct

4.3.1 Description of Study Progress and Visits

At the screening visit—ideally 3 days (or at minimum 2 days) prior to surgery—patients will be approached. After signing the consent form, demographic and medical history questionnaires will be completed. Patients will then be randomized.

Two days before or the day before surgery (t-1), the first blood sample (hs-CRP and ANGPTL2) will be taken, followed by administration of the intervention (quercetin 500 mg BID or placebo).

On the day of surgery (t0), the study medication will be administered before and after surgery.

On the day after surgery (t1), blood tests (hs-CRP and ANGPTL2) will be repeated, as well as on day 4 post-op (t2), and at hospital discharge or up to a maximum of 7 days post-op (t3).

Annex 1 presents a schedule of procedures, study drug administration, and planned lab analyses.

4.3.2 Description of the Intervention

Details of the intervention are provided in Annex 1.

Note: Study medication must be discontinued if a quinolone is prescribed postoperatively. However, to follow an intent-to-treat (ITT) approach, blood tests for hs-CRP and ANGPTL2 should still be performed during hospitalization even if the patient has stopped taking the study medication.

4.3.3 Description of Randomization

Patients will be randomly assigned to one of the two study groups using a randomization table (quercetin 500 mg BID vs. placebo).

4.4 Measurements and Data Collection Methods

To characterize the study population, a demographic questionnaire will be administered. The following data will be collected:

- Age
- Sex
- Preoperative weight
- Height
- Risk factors:
    - Dyslipidemia
    - Hypertension
    - Obesity
    - Diabetes
    - Active smoking
    - Chronic obstructive pulmonary disease
    - Family history of atherosclerotic heart disease
- Date of prior myocardial infarction
- Acute renal failure

Preoperative medications:

- Beta-blockers
- ACE inhibitors
- Angiotensin II receptor blockers
- Diuretics
- Antidiabetic drugs
- Anticoagulants
- Aspirin
- Statins

Hemodynamic values:

- Blood pressure
- Heart rate

Blood analyses:

- Total cholesterol
- LDL (low-density lipoproteins)
- HDL (high-density lipoproteins)

- Triglycerides
- HbA1c (glycated hemoglobin)
- Creatinine
- Estimated glomerular filtration rate (eGFR)

In addition, at specified time points:

- hs-CRP measurement
- ANGPTL2 measurement

These lab tests may be combined with standard pre- and post-operative bloodwork protocols.

To evaluate the senolytic effect of quercetin on endothelial cells:

- Ex vivo acetylcholine-induced endothelial relaxation measurement;
- mRNA quantification in isolated arterial wall cells.

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5. Statistical Aspects

5.1 Sample Size Calculation

The primary evaluation criterion for this study is the change in hs-CRP blood levels between baseline (t-1) and four days after surgery (t2). A logarithmic transformation of the data is planned prior to analysis, and the sample size calculation was based on this assumption.
Given this log transformation, the change between baseline and t2, as well as the difference between groups, is expressed as a **ratio**. Based on data from a previous study, the assumed standard deviation for the log-transformed hs-CRP ratio (hs-CRP at t2 / hs-CRP at t-1), common to both groups, is 1.215 (1).
A sample of 50 subjects per group (100 subjects total) will allow detection of a 50% relative reduction in the change in hs-CRP in the quercetin group compared to the placebo group, with a two-sided significance level of 0.05 and a power of 80%.
A 50% reduction in hs-CRP change would represent a clinically meaningful improvement.

5.2 Statistical Analyses

5.2.1 General Considerations

Descriptive statistics will be provided for all study variables for the total sample and by intervention group. Means, standard deviations, medians, quartiles, minimums, and maximums will be used for continuous variables, while categorical variables will be described using frequencies and percentages.
All assumptions underlying the planned analyses will be verified first. All statistical tests will be two-tailed with a significance threshold of 0.05. No imputation will be performed for missing

values, and no adjustments will be made for multiple testing. Statistical analysis will be conducted using **SPSS software version 25**.

5.2.2 Statistical Analyses

A logarithmic transformation is planned for hs-CRP values prior to analysis. The change in hs-CRP levels from baseline (t-1) will be compared between groups using **repeated measures ANCOVA** including terms for:

- Intervention group (quercetin vs. placebo),
- Time,
- Group × Time interaction,
- Baseline adjustment (t-1).

The comparison between groups at day 4 post-surgery (t2) will address the **primary objective**. Comparisons at other time points will be considered **exploratory**.

The change in ANGPTL2 levels will be analyzed in the same way as hs-CRP: repeated measures ANCOVA including group, time, interaction, and baseline adjustment.

The **endothelial relaxation function induced by acetylcholine (ex vivo)** and **mRNA expression assays** in arterial wall cells will be compared between groups using **Student's t-tests**.

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6. References

1. Noly PE, Labbe P, Thorin-Trescases N et al. *Reduction of plasma angiopoietin-like 2 after cardiac surgery is related to tissue inflammation and senescence status of patients*. J Thorac Cardiovasc Surg 2019;158:792–802.e5.
2. Andres S, Pevny S, Ziegenhagen R et al. *Safety Aspects of the Use of Quercetin as a Dietary Supplement*. Mol Nutr Food Res 2018;62.
3. Ou Q, Zheng Z, Zhao Y, Lin W. *Impact of quercetin on systemic levels of inflammation: a meta-analysis of randomized controlled human trials*. Int J Food Sci Nutr 2020;71:152–163.
4. Bhaskar S, Kumar KS, Krishnan K, Antony H. *Quercetin alleviates hypercholesterolemic diet-induced inflammation during progression and regression of atherosclerosis in rabbits*. Nutrition 2013;29:219–29.
5. Egert S, Wolffram S, Schulze B et al. *Enriched cereal bars are more effective in increasing plasma quercetin compared with quercetin from powder-filled hard capsules*. Br J Nutr 2012;107:539–46.
6. Boots AW, Haenen GR, Bast A. *Health effects of quercetin: from antioxidant to nutraceutical*. Eur J Pharmacol 2008;585:325–37.

7. Wang L, Wang B, Li H et al. *Quercetin, a flavonoid with anti-inflammatory activity, suppresses the development of abdominal aortic aneurysms in mice*. Eur J Pharmacol 2012;690:133–41.
8. Mariee AD, Abd-Allah GM, El-Beshbishy HA. *Protective effect of dietary flavonoid quercetin against lipemic-oxidative hepatic injury in hypercholesterolemic rats*. Pharm Biol 2012;50:1019–25.
9. Marunaka Y, Marunaka R, Sun H et al. *Actions of Quercetin, a Polyphenol, on Blood Pressure*. Molecules 2017;22.
10. Eid HM, Haddad PS. *The Antidiabetic Potential of Quercetin: Underlying Mechanisms*. Curr Med Chem 2017;24:355–364.
11. Patel RV, Mistry BM, Shinde SK, Syed R, Singh V, Shin HS. *Therapeutic potential of quercetin as a cardiovascular agent*. Eur J Med Chem 2018;155:889–904.
12. Sharp MA, Hendrickson NR, Staab JS, McClung HL, Nindl BC, Michniak-Kohn BB. *Effects of short-term quercetin supplementation on soldier performance*. J Strength Cond Res 2012;26 Suppl 2:S53–60.
13. McAnulty SR, McAnulty LS, Nieman DC et al. *Chronic quercetin ingestion and exercise-induced oxidative damage and inflammation*. Appl Physiol Nutr Metab 2008;33:254–62.
14. Nieman DC, Henson DA, Davis JM et al. *Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA*. J Appl Physiol (1985) 2007;103:1728–35.
15. Zahedi M, Ghiasvand R, Feizi A, Asgari G, Darvish L. *Does Quercetin Improve Cardiovascular Risk Factors and Inflammatory Biomarkers in Women with Type 2 Diabetes: A Double-blind Randomized Controlled Clinical Trial*. Int J Prev Med 2013;4:777–85.
16. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. *Quercetin reduces blood pressure in hypertensive subjects*. J Nutr 2007;137:2405–11.
17. Dabeek WM, Marra MV. *Dietary Quercetin and Kaempferol: Bioavailability and Potential Cardiovascular-Related Bioactivity in Humans*. Nutrients 2019;11.

ANNEX 1 – Timeline for Study Medication Administration and Blood Sampling

t-2 (2 days before surgery) - Screen hospitalized patients for eligibility

Present study and obtain consent

Administer demographic questionnaire

Randomization
t-1 (Day before surgery) 06:00 Blood tests (hs-CRP, ANGPTL2)
09:00 Study drug administration
21:00 Study drug administration
t0 (Day of surgery) 06:00 or 09:00 Study drug (depending on surgical schedule)
During surgery Collection of mammary artery segment
21:00 Study drug administration
t1 (Post-op Day 1) 09:00 Study drug administration
18:00 Blood tests (hs-CRP, ANGPTL2)
21:00 Study drug administration
Day 2 09:00 / 21:00 Study drug administration
Day 3 09:00 / 21:00 Study drug administration
t2 (Post-op Day 4) 06:00 Blood tests (hs-CRP, ANGPTL2)
09:00 / 21:00 Study drug administration
Day 5 09:00 / 21:00 Study drug administration
Day 6 09:00 / 21:00 Study drug administration
t3 (Discharge or max Day 7) 06:00 Blood tests (hs-CRP, ANGPTL2)
09:00 Final dose of study drug

Note: Study medication will be administered daily throughout hospitalization, up to a maximum of 7 days post-surgery or until discharge.