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(54) Title: INTRACORONARY ACETYLCHOLINE FORMULATION FOR TESTING CARDIAC CONDITIONS

(57) Abstract: Dosage formulations of acetylcholine in sterile packaged units for injection into a catheter for delivery in a coronary artery are provided. These are selected from ACH doses of 2µg, 20µg, 50µg, 100µg, and 200µg compounded and diluted in sterile saline or phosphate buffered saline for direct bolus injection into the human coronary artery. These are injected into the coronary artery to diagnose whether or not a patient has coronary vasospasm.



INTRACORONARY ACETYLCHOLINE FORMULATION FOR TESTING CARDIAC CONDITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of and priority to U.S. Provisional
Application No. 63/594,167 filed October 30, 2023, which is hereby
incorporated by reference in its entirety.

FIELD OF THE INVENTION

 The invention is generally in the field of intracoronary acetylcholine dosage formulations
10 for testing for ischemic heart disease.

BACKGROUND OF THE INVENTION

 Chest pain is one of the most common presenting symptoms for patients who seek
medical care. Nearly half of people with cardiac chest pain (related to a lack of blood flow to the
heart), will have a coronary vasomotor disorder, or “ischemia and no obstructive coronary artery
15 disease (INOCA).” Current professional society guidelines (2021 ACC/AHA Chest Pain; 2019
ESC Chronic Coronary Syndrome) recommend additional testing to characterize the underlying
diagnosis (including coronary microvascular dysfunction or coronary vasospasm). However,
comprehensive assessments of coronary physiology (including the epicardial vessels and
microvascular bed) are currently not performed routinely in most medical centers due to existing
20 protocols that are overly time consuming and require specialized equipment. As a result, the
majority of patients with INOCA do not undergo guideline- recommended testing. This can lead
to misdiagnosis and incorrect treatment.

 Technological advances allow diagnosis of abnormalities in coronary physiology that
drive symptoms in many patients using validated invasive assessments of coronary artery
25 pressure and flow. Unfortunately, adoption of invasive physiological testing remains low due to
the increased effort and expertise required and a perceived limited clinical value in the absence of
targeted therapies.

 It is therefore an object of the present invention to provide formulations and a protocol
for diagnosis and treatment of patients with chest pain not resulting from blocked blood vessels.

30 It is a further object of the present invention to provide methods of use, and interpretation
of data obtained thereby, for the diagnosis and treatment of individuals with coronary
vasospasm, which may occur more frequently in women.

BRIEF SUMMARY OF THE INVENTION

Dosage formulations of acetylcholine (ACH) in sterile packaged units for injection into a catheter for delivery in a coronary artery are provided. These are selected from ACH doses of 2 μ g, 20 μ g, 50 μ g, 100 μ g, and 200 μ g compounded and diluted in sterile saline or phosphate buffered saline for direct bolus injection into the human coronary artery. In the preferred embodiments, these are in a luer lock syringe having a capacity of between one and three milliliters. The essential dosages in the sterile packaged units are 20 μ g, 100 μ g, and 200 μ g ACH. In the preferred embodiment, the dosage is provided in a volume of 2 mls.

Drug Dose	Volume Administered	Concentration
2 μ g	2mL	1 μ g/mL
20 μ g	2mL	10 μ g/mL
50 μ g	2mL	25 μ g/mL
100 μ g	2mL	50 μ g/mL
200 μ g	2mL	100 μ g/mL

These are injected into the coronary artery to diagnose whether or not a patient has coronary vasospasm or coronary endothelial dysfunction.

Starting dose: The administration of low-dose ACH (2 μ g to 20 μ g) is important for two reasons. First, it allows for evaluation of endothelial function without the induction of spasm (typically seen at higher doses). In most patients the recommended starting dose of ACH is 20 μ g and the typical highest dose is 100 μ g of ACH, although an evaluation of the graded effect of ACH administration in producing spasm demonstrated that there was a modest increase in the incidence of spasm in men at 200 μ g compared with 100 μ g. In the event that the RCA is chosen for provocative testing, it is generally recommended to not administer more than 50 μ g of ACH due to a higher risk of arrhythmia. The fundamental purpose of ACH testing is to evaluate endothelial function and test for coronary vasospasm. Although the total number of doses administered varies among institutional protocols, two doses are generally sufficient to complete this assessment.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic of the method: patient with suspected ischemia, ACH testing, then guidewire testing: FFR/CFR/IMR. Fractional flow reserve (FFR) is a ratio of coronary and aortic pressures characterizing epicardial conductance. Coronary flow reserve (CFR) assesses both the epicardial and microvascular compartments and is defined as the ratio between hyperemic and resting flows. PressureWire X guidewire (Abbott Vascular) is a wireless physiology pressure wire that enables measurement of pressure and temperature to calculate: Resting Full-Cycle Ratio (RFR), FFR and Index of Microcirculatory Resistance (IMR).

FIG. 2 is a schematic of the decision tree based on response to ACH to diagnose the cause of angina or other defects characteristic of non-obstructive coronary arterial disease.

FIG. 3 is a schematic of coronary ACH coronary reactivity to the formulations described herein and the resulting diagnosis.

FIG. 4 is an image of the five dosage units.

DETAILED DESCRIPTION OF THE INVENTION

The ACH dosage formulations and methods of use thereof is based on two discoveries: that many patients presenting with symptoms of a heart attack do not have obstructive coronary artery disease, but distinct disorders requiring vastly different treatment; and ACH dosage formulations that can be administered via cardiac catheterization can rapidly, safely and effectively diagnose conditions such as endothelial dysfunction or coronary vasospasm requiring treatment other than stents or angioplasty.

The coronary vasculature comprises of epicardial arteries ($>400\text{ }\mu\text{m}$), pre-arterioles ($100\text{--}400\text{ }\mu\text{m}$), arterioles ($<100\text{ }\mu\text{m}$) and capillaries ($<10\text{ }\mu\text{m}$). The epicardial arteries function as capacitance vessels and respond to shear forces by endothelium-mediated dilatation. Epicardial arteries are visible on coronary angiography but represent only 5–10% of the coronary vasculature. The pre-arterioles, arterioles and capillaries form the coronary microvasculature. At each level, vessels are lined with endothelium, which plays an important role in the modulation of vascular tone by synthesizing and releasing several vasodilator substances, such as nitric oxide (NO). Increased endothelial wall shear stress and ACH are determinants of coronary blood flow (CBF) in health. Both lead to the biosynthesis of NO, which acts on the neighboring smooth muscle cells to induce vasodilation via the NO pathway.

There is extensive safety data regarding the safety of intracoronary ACH administration (Takahashi et al *J Am Coll Cardiol.* 2022 Jun, 79 (24) 2367–2378. However, due to the complexity of compounding (Figure 2), ACH testing is not routinely performed in more than 95% of the cardiac catheterization laboratories in the United States.

5 **I. Definitions**

Coronary artery disease:

Obstructive coronary artery disease (“CAD”). This type occurs when a fatty substance called plaque builds up the coronary arteries, leading to artery narrowing. It's the most common type of coronary heart disease.

10 Ischemia and no obstructive coronary artery disease (“INOCA”). There is no or minimal plaque buildup in the coronary arteries. Instead, narrowing in the coronary artery is caused by other conditions, including inappropriate constrictions (coronary vasospasm), heart tissue that compresses the coronary arteries (myocardial bridging), damage to the artery lining (endothelial dysfunction), and impaired opening of the microvascular vessels.

15 Coronary blood flow is referred to as “CBF”.

Coronary microvascular disease (“CMD”) is generally used to indicate abnormal findings during adenosine testing, and indicates problems in the non-endothelium dependent function of the microvasculature of the coronary arteries.

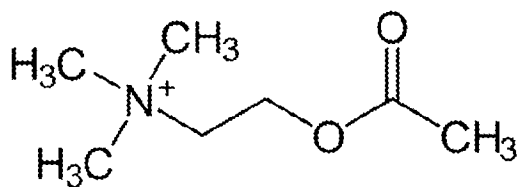
20 Coronary flow reserve (“CFR”). CFR represents the ratio of hyperemic to baseline coronary blood flow (CBF), i.e., the capacity of the coronary circulation to deal with increased demand for myocardial perfusion, similar to a “stress test”. There are two important limitations to measuring CFR. The first is that it incorporates the function of the epicardial vessel and hence is not specific to the microvasculature. Second, CFR is a ratio that is dependent on the baseline CBF, and there is significant variability in resting blood flow, which can make it challenging to
25 interpret CFR as it pertains to microvascular function.

II. Compositions

A. Acetylcholine

Acetylcholine (“ACH”) is an ester of acetic acid and choline, which acts as a neurotransmitter.

30



ACH is the chief neurotransmitter of the parasympathetic nervous system, the part of the autonomic nervous system. a branch of the peripheral nervous system that contracts smooth muscles, dilates blood vessels, increases bodily secretions, and slows heart rate. ACH can stimulate a response or block a response and thus can have excitatory or inhibitory effects.

ACH has dual effects on coronary arteries. It binds to the muscarinic 3 (M3) receptor on endothelial cells and leads to an influx of intracellular calcium (Ca^{2+}) via the L-type calcium channels. Intracellular Ca^{2+} binds to the protein calmodulin, and the calcium-calmodulin complex activates the endothelial nitric oxide synthase (eNOS) enzyme, which catalyses the conversion of L-arginine into nitric oxide (NO). NO then diffuses into the neighbouring vascular smooth muscle cell (VSMC) and activates guanylate cyclase (GC) enzyme to catalyse the conversion of guanosine triphosphate (GTP) into cyclic GMP (cGMP). cGMP activates the protein kinase G (PKG), which, via a series of intracellular events, inactivates the calcium channels on the VSMC. This reduces the intracellular influx of Ca^{2+} into the VSMC, therefore leading to vasodilation. ACH also binds to the M3 receptor on the surface of VSMCs and, in the presence of endothelial dysfunction, leads to unopposed vasoconstriction. Endothelin-1 (ET-1) binds to its receptor (ETA) and activates Rho-kinase, which inhibits MLCP and leads to vasoconstriction. VSM relaxation, and therefore vasodilation, occurs when there is reduced phosphorylation of MLC. This can result from reduced intracellular Ca^{2+} concentration, inhibition of MLCK by increased intracellular concentration of cAMP and MLCP-activated MLC dephosphorylation.

ACH chloride currently is only available as an intraocular solution, methacholine chloride is available as a powder, bethanechol chloride is available as tablets, and carbachol is available as an ophthalmic solution. MIOCHOL[®]-E (acetylcholine chloride intraocular solution) is a parasympathomimetic preparation for intraocular use. It is packaged in a blister pack containing one vial and one ampoule. The vial contains 20 mg acetylcholine chloride and 56 mg mannitol. The accompanying ampoule contains 2 mL of a modified diluent of sodium acetate trihydrate, potassium chloride, magnesium chloride hexahydrate, calcium chloride dihydrate and sterile water for injection.

The reconstituted liquid is a sterile isotonic solution (275–330 milliosmoles/Kg) containing 20 mg acetylcholine chloride (1:100 solution) and 2.8% mannitol. The pH range is 5.0–8.2.

<https://www.bausch.com/globalassets/pdf/packageinserts/pharma/miochol-e-acetylcholine-chloride-intraocular-solution.pdf>

B. Reformulation into dosage units.

ACH can be reformulated into dosage units using sterile ACH. However, since the only Food and Drug Administration approved ACH is the ophthalmic ACH, in the preferred embodiment the ophthalmic ACH (Bausch and Lomb MIOCHOL®) is compounded into sterile pre-filled syringes which can be stored and delivered to the cardiac catheterization laboratory for each procedure.

Currently, coronary artery administration of ACH is an off-label use of an ophthalmic medication, and after reconstitution of a powder, each facility must make serial dilutions so that it can be safely injected into the coronary arteries. This must be performed for each individual patient that undergoes testing, placing a strain on hospital resources and staff. Stability testing data from Cedars-Sinai Medical Center (Los Angeles, CA) and facilities outside of the United States has shown that after compounding, ACH remains effective for a minimum of 7 days or up to 28 days. Access to commercially available pre-filled ACH syringes drastically reduces the time and complexity required to perform invasive coronary function testing (CFT). As a result, this testing can be scaled to other hospitals.

These dosage formulation units preferably are provided as a disposable 5-chamber storage and delivery system for the diagnostic drug. These units are characterized by being a single use, sterile system, which can be refrigerated for at least one month, with five separate dosage chambers (2 to 3mL per chamber, maximum of 5mL), with appropriate labeling, where volume is readily accessible, and which is compatible with standard male luer lock syringe (e.g., 10mL male luer lock syringe). The disclosed dosage forms provide an additional advantage in that the acetylcholine therein is stable for at least and up to 28 days reducing the burden on hospital resources and staff. For example, the dosage forms are stable for at least 5, 10, 15, 20, 25, and up to about 28 days. Stability can be determined in some forms, where the solution is clear and colorless. Methods for determining acetylcholine stability over time are known in the art (Sletten, et al., *Journal of the Neurological Sciences*, Volume 234, Issue 1, 1 – 3 (2005)). In sum, the stability of acetylcholine in solution can be determined by reverse-phase HPLC with

electrochemical detection using an Acetylcholine/Choline Assay Kit. The inherent levels of Ch were used as the internal standard.

The reconstituted ophthalmic ACH is a sterile isotonic solution (275–330 milliosmoles/kg) containing 20 mg acetylcholine chloride (1:100 solution) and 2.8% mannitol.

5 The pH range is 5.0–8.2.

The dosage unit formulations which have been developed are provided in five ACH doses (2µg, 20µg, 50µg, 100µg, and 200µg) compounded and diluted in sterile saline for direct bolus injection into the human coronary artery.

10 Other ACH formulations that have been described for coronary use are neither these dosages, nor in a form that can be used for injection into the coronary artery. For example, Scuri et al., *J Appl Physiol* (1985). 1996 Jan;80(1):341-44. doi: 10.1152/jappl.1996.80.1.341. PMID: 8847325 describes a nebulized aerosolized formulation; Crippa et al. *Cereb Cortex*. 1999 Jun;9(4):362-5. doi: 10.1093/cercor/9.4.362. PMID: 10426415 describes nanomolar dosing in saline with direct injection into the cerebral cortex; Phillips, et al. *The British Medical Journal* 1, 15 no. 4877 (1954): 1468–70. <http://www.jstor.org/stable/20329271> describes milligram dosing, up to 100mg, not microgram dosing; and Francis et al. *Amer, Heart J*. 1960 60(3):440-443, also describes milligram dosing in an aqueous mannitol solution.

There is extensive safety data regarding the safety of intracoronary ACH administration (summarized here: <https://www.jacc.org/doi/abs/10.1016/j.jacc.2022.03.385>). However, due to 20 the complexity of compounding, ACH testing is not routinely performed in > 95% of the cardiac catheterization laboratories in the United States. Chest pain is one of the most common presenting symptoms for patients who seek medical care. Nearly half of people with cardiac chest pain (related to a lack of blood flow to the heart), will have a coronary vasomotor disorder, or “ischemia and no obstructive coronary artery disease (INOCA).” Current professional society 25 guidelines (2021 ACC/AHA Chest Pain; 2019 ESC Chronic Coronary Syndrome) recommend additional testing to characterize the underlying diagnosis (including coronary microvascular dysfunction or coronary vasospasm).

C. Acetylcholine Dosage Units

30 Dosage units consist of five 5 pre-diluted ACH doses: 2µg, 20µg, 50µg, 100µg, and 200µg, compounded and diluted in sterile saline for direct bolus injection into the human coronary artery.

As described herein, the package for use in testing patients will include at least three different dosages, optionally five dosages. In most clinical practice only two or three of the

dosages are used. The five doses are: 2µg, 20µg, 50µg, 100µg, and 200µg. The essential doses are 20µg, 100µg, and 200µg.

Preparation by Dilution into Single Syringe Dosage Unites

Ophthalmological ACH vial contains 20 mg un-reconstituted acetylcholine chloride powder. Reconstitute powder with 2 ml sterile diluent for 20 mg/2 ml concentrated solution. Desired concentrations for intracoronary administration are:

- Syringe 1: 2 µg/2 ml (1 µg/ml) Dose 2 µg
- Syringe 2: 20 µg/2 ml (10 µg/ml) Dose 20 µg
- Syringe 3: 50 µg/2 ml (25 µg/mL) Dose 50 µg
- 10 Syringe 4: 100 µg/2 ml (50 µg/ml) Dose 100 µg
- Syringe 5: 200 µg/2 ml (100 µg/ml) Dose 200 µg

All dilutions are in sterile saline or sterile phosphate buffered saline.

syringe 1 (2 µg) (optional):

- a. withdraw 0.1 ml of 20 mg/2ml reconstituted ACH solution using a 1 cc syringe
 - 15 b. inject 0.1 ml of concentrated ACH into a 1 L bag of normal saline
 - c. withdraw 2 ml from 1 L bag into a 3 cc syringe for a 2 µg/2 ml final concentration
- (10 mg/ml x 0.1 ml = 1 µg/ml x 1000 ml)

syringe 2 (20 µg):

- a. withdraw 0.1 ml of 20 mg/2ml reconstituted ACH solution using a 1 cc syringe
 - 20 b. inject 0.1 ml of concentrated ACH into a 100 mL bag of normal saline
 - c. withdraw 2 ml from 100 ml bag into a 3 cc syringe for a 20 µg/2 ml final
- concentration (10 mg/ml x 0.1 ml = 10 µg/ml x 1000 ml)

syringe 3 (50 µg):

- a. withdraw 0.5 ml of 20 mg/2ml reconstituted ACH solution using a 1 cc syringe
 - 25 b. inject 0.5 ml of concentrated ACH into a 200 mL bag of normal saline
 - c. withdraw 2 ml from 200 ml bag into a 3 cc syringe for a 50 µg/2 ml final
- concentration (10 mg/ml x 0.5 ml = 25 µg/ml x 1000 ml)

syringe 4 (100 µg):

- a. withdraw 0.5 ml of 20 mg/2ml reconstituted ACH solution using a 1 cc syringe
 - 30 b. inject 0.5 ml of concentrated ACH into a 100 mL bag of normal saline
 - c. withdraw 2 ml from 100 ml bag into a 3 cc syringe for a 100 µg/2 ml final
- concentration (10 mg/ml x 0.5 ml = 50 µg/ml x 1000 ml)

syringe 5 (200 µg):

- a. withdraw 1 ml of 20 mg/2ml reconstituted ACH solution using a 1 cc syringe
- b. inject 1 ml of concentrated ACH into a 100 mL bag of normal saline
- c. withdraw 2 ml from 100 ml bag into a 3 cc syringe for a 200 µg/2 ml final concentration ($10 \text{ mg/ml} \times 1 \text{ ml} = 50 \text{ µg/ml} \times 1000 \text{ ml}$)

Alternatively, one can draw up from the 20 µg/ml solution:

1 ml: 20 µg

2.5 ml: 50 µg

5 ml: 100 µg

5 10 ml: 200 µg

In another embodiment, the ACH can be prepared by dissolution of the ACH into sterile saline or phosphate buffered saline, if pharmaceutically acceptable ACH which has been produced under an appropriate GMP protocol is available.

III. Methods of Diagnosis and Treatment

10 Ischemic heart disease (IHD) is a leading cause of morbidity and mortality in women and men. The diagnostic pathway for suspected IHD involves stress testing and coronary angiography. Coronary angiography (or cardiac catheterization) is the gold-standard for diagnosing stable IHD and evaluating patients with suspected myocardial infarction (MI) because the etiology is often obstructive coronary artery disease (CAD). However, half of
15 patients with stable symptoms have ischemia with no evidence of obstructive CAD (INOCA) and 25% with an acute MI have no evidence of obstructive plaque or thrombosis during coronary angiography (MINOCA). These syndromes encompass a heterogeneous pathophysiology, including coronary vasospasm and microvascular dysfunction, which are not identified by routine coronary angiography. As a result, many patients who undergo cardiac catheterization for
20 suspected IHD are not given a specific diagnosis or a targeted therapeutic plan, contributing to ongoing symptoms, recidivism, and poor outcomes.

Coronary microvascular dysfunction (CMD) is a type of non-obstructive coronary artery disease that causes the small blood vessels feeding the heart muscle to not work as they should. This can result in discomfort in the chest that can feel like a heaviness, tightness, pressure or
25 squeezing, sweating, nausea and dizziness, stomach pain, difficulty breathing, being very tired (fatigue) and having low energy.

Despite major advances in diagnosing INOCA, therapeutic strategies for coronary vasospasm remain limited and poorly defined. Treatments may include calcium channel blockers (“CCBs”) and nitrates constitute the treatment of choice. Lifestyle changes such as
30 improving diet, doing regular exercise, not smoking, reducing obesity and controlling diabetes can often improve symptoms.

A comprehensive physiology-guided diagnostic approach can improve the precision of patients with chest pain who present to the catheterization laboratory with suspected IHD.

However, comprehensive assessments of coronary physiology (including the epicardial vessels and microvascular bed) are currently not performed routinely in most medical centers due to existing protocols that are overly time consuming and require specialized equipment. As a result, the majority of patients with INOCA do not undergo guideline- recommended testing and appropriate treatment. See Sinha A, et al. Coronary microvascular disease: current concepts of pathophysiology, diagnosis and management. *Cardiovasc Endocrinol Metab.* 2020 Jul 16;10(1):22-30. doi: 10.1097/XCE.0000000000000223. PMID: 33634252; PMCID: PMC7901821; and Smilowitz, et al.

The following Comprehensive Coronary Physiology Protocol can be performed in less than one hour using equipment that is commercially available, using the dosage formulations described herein. The protocol and specific dosage unit ACH formulations are scalable and performed routinely within the standard of care in this field.

Testing of the Microcirculation and Epicardium with Acetylcholine.

Because the endothelium is present in all-sized coronary vessels, the use of ACH during invasive coronary function testing (CFT) allows for evaluation of both the microcirculatory and epicardial circulation. In the regulation of coronary vasomotor tone, ACH acts on the muscarinic receptors (AChM3R) in both the endothelium and vascular smooth muscle. In coronary arteries with healthy endothelium, ACH induces endothelium- dependent dilation by release of nitric oxide, which predominates over ACH-induced smooth muscle- mediated constriction. In the presence of endothelial dysfunction and impaired release of nitric oxide, smooth muscle- mediated constriction predominates in response to ACH. Higher doses of intracoronary ACH may increase the likelihood of inducing vasoconstriction through direct stimulation of the smooth muscle muscarinic receptors, particularly in the setting of endothelial dysfunction or smooth muscle hyperreactivity. Although endothelial function can be assessed by other means, such as dobutamine, exercise, cold pressor testing, and mental stress, testing with ACH remains the gold standard for invasive testing of endothelial function. The overall prognosis of patients with coronary spasm is favorable; however, patients with epicardial spasm are at increased risk for myocardial infarction and repeat angiography, whereas microvascular spasm is associated with recurrent angina.

Equipment and protocol. Doses of ACH vary widely in the literature and clinical practice, typically ranging from an ultralow dose of 0.36 mg to a high dose of 200 mg. All protocols recommend the administration of graded doses of ACH, monitoring of ECG and clinical symptoms, and performance of coronary angiography in a consistent view between doses

to allow for assessment of epicardial spasm before proceeding to the next dose. In addition, use of metallic letters or numbers on the cine pictures to differentiate doses can be helpful for later or outside review.

Dosing of ACH. Although all centers recommend a graded administration of ACH, there are significant differences in the starting, the highest, and the total number of doses administered.

Starting dose: The administration of low-dose ACH is important for two reasons. First, it allows for evaluation of endothelial function without the induction of spasm (typically seen at higher doses). Second, administration of higher doses can be avoided in the event that spasm does occur at a lower dose, although experience suggests that low-dose ACH administration rarely produces epicardial spasm. Starting dose of ACH at 20 µg is recommended.

Highest dose: Most investigators have used protocols that stopped at the administration of 100 µg of ACH, although some centers have continued to 200 µg in the absence of demonstrated spasm with the 100µg dose. An evaluation of the graded effect of ACH administration in producing spasm demonstrated that there was a modest increase in the incidence of spasm in men at 200 µg compared with 100 µg, although there was no demonstrable increase in spasm among women above the 50 µg dose. In the event that the RCA is chosen for provocative testing, it is generally recommended to not administer more than 50 µg of ACH due to a higher risk of arrhythmia.

Total number of doses administered: The fundamental purpose of ACH testing is to evaluate endothelial function and test for coronary vasospasm. Although the total number of doses administered varies among institutional protocols, two doses are sufficient to complete this assessment. Routine administration of 100 µg has a very low incidence of adverse events. Therefore, after the initial dose, proceeding directly to the 100 µg dose for spasm assessment will expedite the procedure without sacrificing safety. One should stop before the higher dose if spasm has been demonstrated at the lower dose, and one may consider giving an additional higher dose (200 mg) if spasm has not been demonstrated but is highly suspected.

Recommendation: Two doses (20 µg and 100 µg). One dose is sufficient if spasm is demonstrated at the lower dose, and a third dose (200 µg) is optional if spasm is not demonstrated at the higher dose but is strongly suspected.

Rate of ACH administration. Many protocols use a slow, gradual intracoronary infusion, generally over 2 to 3 minutes per dose, whereas others use a more rapid infusion (generally 30-60 seconds).

Recommendation: A low dose (20 µg) to assess endothelial function is given as a slow

infusion/injection over 2 to 3 minutes. A high dose (100 µg) to assess coronary spasm is given as a quicker bolus over 30 to 60 seconds, as tolerated (see comments on the safety of bolus administration in the Avoidance of Complications section later).

Method of ACH administration. Some operators prefer infusing ACH directly through the guide catheter, whereas others prefer administration of ACH selectively into the coronary artery of interest (typically the LAD artery) by using a microcatheter. Theoretically, the infusion selectively into the LAD artery avoids the potential for concomitant left circumflex or left main artery spasm. Conversely, infusion directly through the guide catheter has the advantage of simplicity and avoidance of small potential for unwanted spasm, dissection, or other complication of placement of a microcatheter into a selected vessel. Infusion directly through the guide catheter allows for assessment of the left circumflex artery as well.

Endothelial dysfunction is a failure to observe the expected epicardial vasodilation and increase in coronary flow in response to the administration of ACH. The definition of an abnormal angiographic response varies somewhat in the literature, but a practical definition is any epicardial constriction of >0% but <90% after the administration of ACH (ideally determined by quantitative coronary angiography [QCA]). Although the failure to vasodilate and the presence of mild vasoconstriction can often be identified visually, QCA measurements should be encouraged in borderline cases. Endothelial function can also be more precisely evaluated by measuring the changes in overall CBF after ACH administration (normal: >50% increase from baseline), although this requires Doppler velocity and QCA measurements ($\text{CBF} = \frac{1}{4} \pi (\text{average peak velocity} / 2)^2 (\text{vessel diameter} / 2)^2$). One study found evidence of endothelial dysfunction in over two-thirds of ANOCA patients without evidence of CMD to adenosine or spasm from ACH, thus highlighting the need for careful endothelial assessment in these patients.

Microvascular spasm is more challenging to diagnose. Ideally, one demonstrates a decrease in CBF and an increase in coronary sinus lactate production during ACH administration without epicardial constriction. However, for practical purposes, microvascular spasm is currently defined as ischemic ECG changes and angina in the absence of any visible epicardial constriction. Nonetheless, some combination of chest pain and ECG changes may not always represent diffuse microvascular spasm, and other mechanisms, such as significant endothelial dysfunction, may be involved.

Epicardial coronary spasm is generally considered to be present if there is visible severe constriction of the coronary artery, accompanied by ECG changes indicative of ischemia and clinical onset of angina. The definition of severe constriction has varied in the literature from

75% to 90% stenosis when compared with a normal reference segment, with most publications settling on a >90% narrowing definition.

Prinzmetal (variant) angina is a clinical term referring to chest pain associated with transient ST-segment elevation on the ECG. This typically occurs at rest or with mild exercise and represents recurrent episodes of myocardial ischemia secondary to epicardial or microvascular spasm.

Summary of Administration of Acetylcholine Dosages to the Coronary Artery

Bolus Injection	Initial Dose	Second Dose	Maximum Dose*
Left coronary artery	20µg	100µg	200µg
Right coronary artery	2µg	20µg	50µg

The dosage formulations are administered into the coronary artery using standard coronary artery guide catheters. The site of placement is the right or left coronary artery. Provocative testing should preferentially be performed on the left coronary artery due to an increased risk of bradycardia and tachyarrhythmias with provocative testing of the right coronary artery. The right coronary artery may be assessed in select cases.

In the majority of cases, provocative testing with ACH should be performed after diagnostic angiography and before guidewire-based physiology or intracoronary imaging. However, the sequence of testing are based on operator preference and should be documented according to the angiographic acquisition sequence labeling guidelines.

Patients should be monitored with electrocardiographic monitoring according to the catheterization laboratory routine. Additional monitoring with a cardiac monitor with pacing / defibrillator functionality is recommended.

The ACH dosage should be administered by a gradual bolus hand injection via a 6Fr guide catheter and patients should be monitored for bradycardia, atrioventricular block, tachyarrhythmias, or symptoms such as chest pain. See **FIG. 1**.

Patients should be monitored with electrocardiographic monitoring according to the catheterization laboratory routine. Additional monitoring with a cardiac monitor with pacing / defibrillator functionality is recommended.

After a minimum of 30 seconds the patient should be assessed for any new symptoms and an electrocardiogram should be obtained from the telemetry monitor (ideally 12-lead, if possible).

After each dose a cine angiogram should be performed in the exact same projection as the baseline angiogram. The angiographic view is per the discretion of the operator.

After waiting for at least one minute, the dose should be escalated to Treatment of each patient.

- 5 If the patient experiences chest discomfort, there is > 1mm ST-elevation or > 1mm ST-depression on the EKG, and there is 90% vasospasm of the epicardial vessel, then the test is diagnostic for coronary vasospasm and no further doses should be performed.

This procedure can be used to diagnose the following conditions based on the response to injection:

- 10 Coronary artery vasospasm
 Epicardial coronary artery endothelial dysfunction
 Microvascular coronary artery endothelial dysfunction
 Coronary artery microvascular spasm
 Absence of coronary artery vasospasm

- 15 This is illustrated in **FIG. 2** and **FIG. 3**.

The five dosage units ready for use during the procedure are shown in **FIG. 4**.

Treatment of Patients Based on Diagnosis

Appropriate treatments of the patients are reported in the following table from Comprehensive Management of ANOCA, Part 2—Program Development, Treatment, and

Research Initiatives. J Am Coll Cardiol. 2023 Sep, 82 (12) 1264–1279.

<https://doi.org/10.1016/j.jacc.2023.06.044>, as shown in the following table.

TABLE 1: Summary of CFF Subclass Recommendations							
Guideline	Year	Indications/Contraindications	Class	LOE	Pharmacologic Agents	Protocol	Diagnostic Criteria
ESC chronic coronary syndrome guidelines	2019	1. Simultaneous-based CFR and/or microcirculatory resistance measurements in patients without obstructive CAD	Class IIIa	B	CMR: adenosine Vasopressin acetylsalicylic acid angiotensins	No recommendation	CMR: 1. IMR ≥ 2.5 or 2. CFR ≤ 2.5 Vasopressin: 1. Severe microcirculatory flow 2. Anginal symptoms 3. Ischemic ECG changes
		2. Intracoronary acetylsalicylic acid during angiography in patients for microvascular vasospasm	Class IIIb	B			
		3. Transcatheter Occluder of the LAD, CMR, or PET MPI for noninvasive assessment of CFR	Class IIIb	B			
		4. An intracoronary provocation test should be considered to identify coronary spasm to diagnose the site and cause of spasm	Class IIIa	B			
AHA/ACC/ASE/CHEST/SAHA/SCCT/SCMR chest pain guidelines	2021	1. Invasive LFT for stable chest pain and nonobstructive CAD and at least mild ischemia on imaging	Class 2a	B	CMR: adenosine Vasopressin acetylsalicylic acid	No recommendation	CMR: 1. IMR ≥ 2.5 or 2. CFR ≤ 2.5 Vasopressin: 1. $\geq 80\%$ stenosis 2. Reproduction of chest pain 3. Ischemic ECG changes
		2. PET MPI with myocardial blood flow reserve for stable chest pain and non-obstructive CAD	Class 2a	B			
		3. Stress CMR with myocardial blood flow reserve for stable chest pain and non-obstructive CAD	Class 2a	B			
		4. Stress echocardiography with coronary flow velocity reserve for stable chest pain and nonobstructive CAD	Class 2b	C			
ECG diagnosis and treatment of vasospastic angina	2023	1. Suspected vasospastic angina	I	B	Vasopressin acetylsalicylic acid angiotensins	• Initiation of temporary pacing • Baseline angiography • Provocation of the ICA	Vasospasm: 1. $\geq 90\%$ stenosis 2. Reproduction of chest pain 3. Ischemic ECG changes
		2. Noninvasively diagnosed vasospastic angina unresponsive to medical therapy	IIa	B		• Provocation of the ICA	
		3. Invasively diagnosed vasospastic angina unresponsive to medical therapy	IIb	B		• Provocation of the ICA	
		4. Not recommended to perform pharmacologic coronary spasm provocation tests for patients without symptoms suggestive of vasospastic angina	In two benefits	C		• Final angiogram after coronary artery intervention	
		5. Should not be performed for patients with acute coronary syndromes in whom serious complications from induced coronary spasm are expected (eg, severe cardiac dysfunction, congestive heart failure)	II (harm)	C			

ACC/AHA/ESC/ASE/SAHA/SCCT/SCMR = American Heart Association/American College of Cardiology/American College of Chest Physicians/Society for Academic Emergency Medicine/Society of Cardiovascular Computed Tomography/Society for Cardiovascular Magnetic Resonance; LAD = coronary artery disease; CFR = coronary flow reserve; CFF = coronary function testing; CMR = coronary magnetic resonance; IMR = index of microcirculatory resistance; IMR = index of microcirculatory resistance; ASE = American Society of Echocardiography; LAD = left anterior descending artery; LCA = left coronary artery; LOE = level of evidence; PET MPI = positron emission tomography myocardial perfusion imaging; ICA = right coronary artery.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed method and compositions belong. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present method and compositions, 5 the particularly useful methods, devices, and materials are as described.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the method and compositions described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

I claim:

1. A sterile packaging comprising dosage formulations of acetylcholine in sterile packaged units for injection into a catheter for delivery in a coronary artery, wherein the acetylcholine dosage are 20 μ g, 100 μ g, and 200 μ g.
2. The sterile packaging of claim 1 further comprising acetylcholine dosages of 2 μ g and 50 μ g acetylcholine.
3. The sterile packaging of claim 1 or 2 wherein the dosages are formulated with sterile saline or sterile phosphate buffered saline, optionally comprising mannitol, in a volume between one and three milliliters, preferably 2 milliliters.
4. The sterile packaging of any of claims 1-3 wherein the dosage formulation is configured to connect to and inject the formulation into a catheter suitable for placement in a coronary artery.
5. A method to diagnoses if a patient coronary vasospasm comprising
placing a catheter into a coronary artery, preferably the left coronary artery, injecting the formulation of any of claims 1-4, and assessing the response.
6. The method of claim 5 comprising administering a starting dose of ACH is 20 μ g.

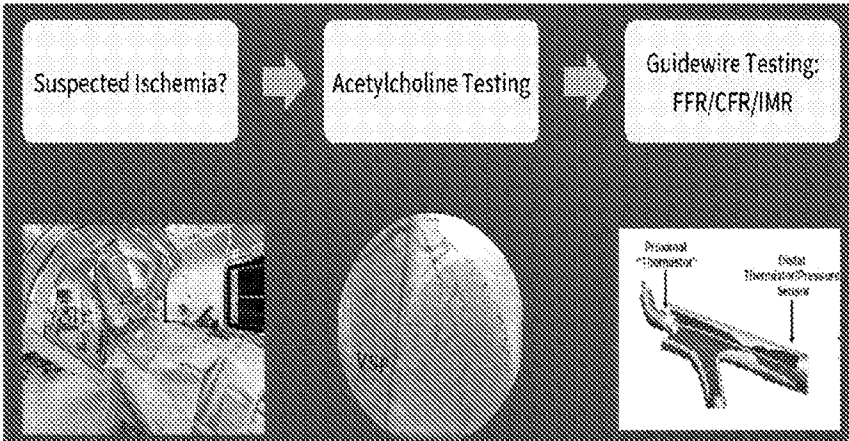


FIG. 1

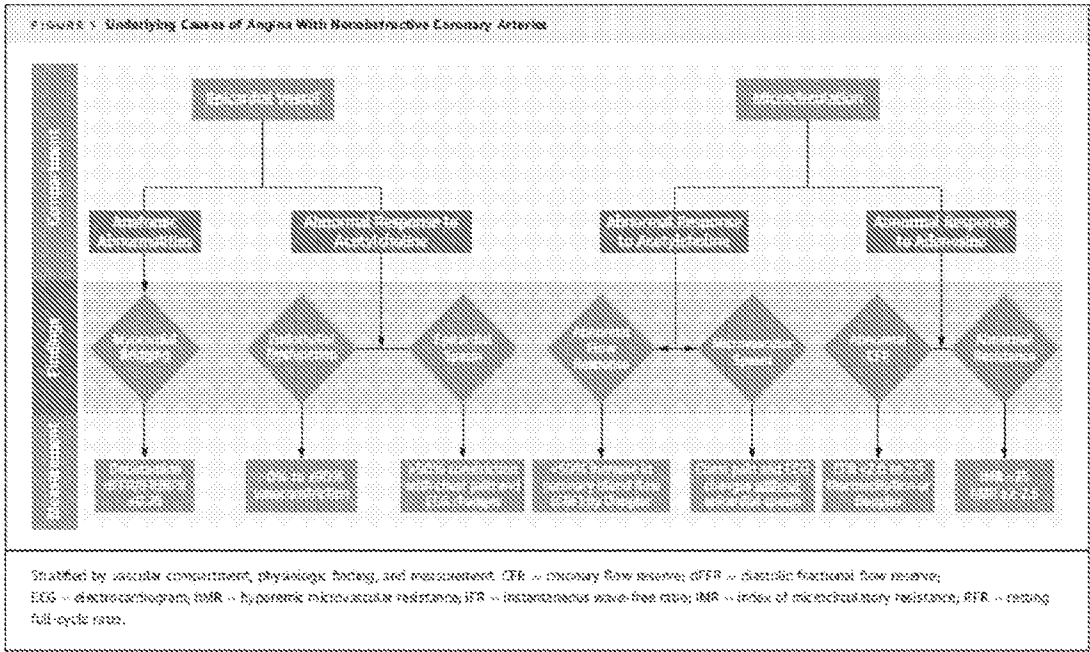


FIG. 2

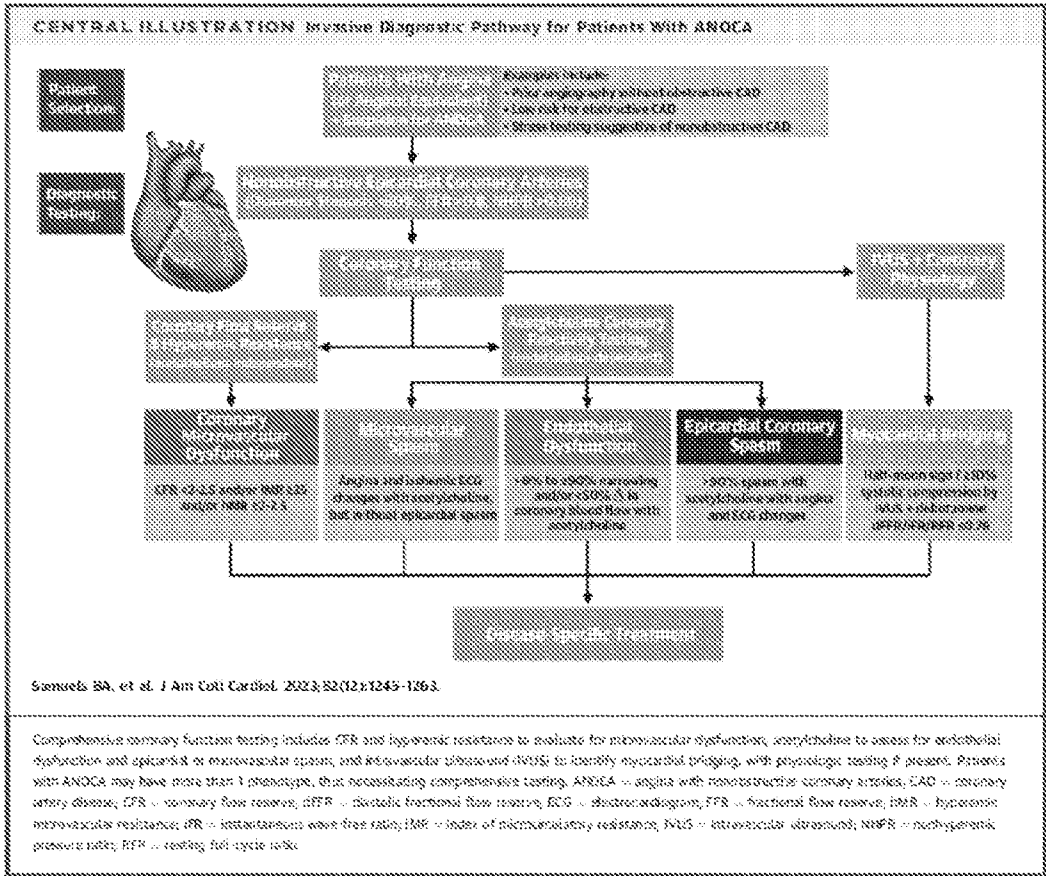


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/053578

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K9/00 A61K31/221 A61P9/10 ADD. A61K9/08		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K A61P Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAMUELS BRUCE A ET AL: "Comprehensive Management of ANOCA, Part 1-Definition, Patient Population, and Diagnosis JACC State-of-the-Art Review", JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 82, no. 12, 11 September 2023 (2023-09-11), pages 1245-1263, XP087396770, ISSN: 0735-1097, DOI: 10.1016/J.JACC.2023.06.043 [retrieved on 2023-09-11] the whole document title tables figures page 1253, right-hand column, last paragraph - page 1257, right-hand column, paragraph 1 - / - -	1 - 6
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 23 January 2025		Date of mailing of the international search report 10/02/2025
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Marchand, Petra

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/053578

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	----- SMILOWITZ NATHANIEL R ET AL: "Comprehensive Management of ANOCA, Part 2-Program Development, Treatment, and Research Initiatives JACC State-of-the-Art Review", JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 82, no. 12, 11 September 2023 (2023-09-11), pages 1264-1279, XP087396936, ISSN: 0735-1097, DOI: 10.1016/J.JACC.2023.06.044 [retrieved on 2023-09-11] the whole document tables figures	1 - 6
X	----- TAKAHASHI TATSUNORI ET AL: "Safety of Provocative Testing With Intracoronary Acetylcholine and Implications for Standard Protocols", JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 79, no. 24, 13 June 2022 (2022-06-13) , pages 2367-2378, XP087096181, ISSN: 0735-1097, DOI: 10.1016/J.JACC.2022.03.385 [retrieved on 2022-06-13] the whole document table 3	1 - 6
A	----- Anonymous: "Summary of Product Characteristics: Miochol -E, 20mg, Powder and Solvent for instillation solution for intraocular use", , 1 June 2014 (2014-06-01), XP093242562, Retrieved from the Internet: URL:https://www.medicines.ie/medicines/miochol-e-20mg-powder-and-solvent-for-solution-for-intraocular-irrigation-32896/spc the whole document section 2 section 6.1 ----- -/-	1 - 6

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/053578

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Anonymous: "Miochol (TM) -E (acetylcholine chloride intraocular solution) 20 mg/2 mL (10 mg/mL)", , 1 March 2023 (2023-03-01), XP093242787, Retrieved from the Internet: URL:https://www.bausch.com/globalassets/pdf/ packageinserts/pharma/miochol-e-acetylch oline-chloride-intraocular-solution.pdf the whole document page 1, line 9 - line 16 -----	1 - 6