

RESEARCH MONOGRAPH . COMPOUND PROFILE

Cardiogen

A synthetic tetrapeptide (H-Ala-Glu-Asp-Arg-OH; AEDR) developed at the Saint Petersburg Institute of Bioregulation and Gerontology as an ultrashort peptide bioregulator with cardioprotective, anti-apoptotic, and epigenetic chromatin-regulatory activity targeting cardiomyocytes, cardiac fibroblasts, and myocardial gene expression programs.

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ABSTRACT

Cardiogen (H-Ala-Glu-Asp-Arg-OH; AEDR tetrapeptide; molecular formula $C_{18}H_{31}N_7O_9$; molecular weight 489.48 g/mol) is a synthetic tetrapeptide bioregulator developed by Vladimir Khavinson and colleagues at the Saint Petersburg Institute of Bioregulation and Gerontology as the cardiac-specific member of the Khavinson ultrashort peptide bioregulator family [1, 2]. The compound belongs to a class of synthetic two-to-seven-residue peptide sequences modeled on tissue-specific peptide fragments isolated from mammalian organ extracts, and is designated as the cardiovascular system bioregulator within this peptide family. Cardiogen shares the Ala-Glu-Asp tripeptide core with the cortical bioregulator Cortagen (Ala-Glu-Asp-Pro) and the pineal bioregulator Epithalon (Ala-Glu-Asp-Gly), differing from these compounds by the fourth-position arginine residue, a single amino acid substitution that determines cardiac tissue specificity within the Khavinson classification system [3, 4]. The principal molecular mechanism of Cardiogen, characterized through molecular modeling, cell culture, and organotypic myocardial tissue studies, is epigenetic regulation of gene expression through direct interaction of the tetrapeptide with double-stranded DNA in gene promoter regions and with histone proteins (H1, H2B, H3, H4), producing chromatin decondensation and reactivation of transcriptional programs in cardiac cells [5, 6, 7]. The cardioprotective activity, characterized in organotypic myocardial tissue cultures, embryonic fibroblast cultures, and coronary artery ligation animal models, includes stimulation of cardiomyocyte proliferation with concurrent suppression of cardiomyocyte apoptosis through p53 protein downregulation, upregulation of

cytoskeletal proteins (actin, vimentin, tubulin) by up to five-fold and nuclear matrix proteins (lamin A, lamin C) by up to 2.5-fold relative to control, preservation of myocardial glycogen stores and cellular energy production structures under ischemic conditions, and a reported threefold reduction in mortality following experimental coronary artery ligation in treated versus control groups [8, 9, 10, 11]. In a separate line of investigation, Cardiogen demonstrated tumor-modifying activity against transplanted M-1 sarcoma in senescent rats, with dose-dependent inhibition of tumor growth mediated by hemorrhagic necrosis and stimulation of tumor cell apoptosis through a vascular mechanism rather than direct cytostatic effect [12]. The compound has been characterized in the context of the senescence-associated secretory phenotype of cardiovascular system cells and inflammaging, with evidence that the AEDR tetrapeptide regulates molecules involved in the inflammatory pathways contributing to age-related cardiovascular decline [13]. No formal pharmacokinetic studies have been published for Cardiogen as the isolated synthetic AEDR tetrapeptide. As a linear tetrapeptide with unprotected termini, the compound is expected to undergo rapid proteolytic degradation by aminopeptidases and carboxypeptidases in plasma and gastrointestinal fluid; however, molecular modeling studies have demonstrated that ultrashort peptides are substrates of the proton-coupled oligopeptide transporter (PEPT1/PEPT2) family carriers, supporting intestinal absorption and cellular uptake through active transport mechanisms [14, 15]. No human clinical trials have been published. The compound is not approved by the United States Food and Drug Administration, the European Medicines Agency, or any major Western regulatory authority. Cardiogen is registered in the Russian Federation as a biologically active additive and is commercially available there in capsule and

sublingual formulations. It is supplied internationally as a research-grade lyophilized peptide by multiple peptide synthesis vendors at greater than 95 percent purity by high-performance liquid chromatography. This monograph reviews the chemistry, synthesis, and structural characterization of Cardiogen; the discovery and development history within the Khavinson bioregulatory peptide program; the molecular pharmacology including peptide-DNA binding, histone interaction, and cardiac gene expression modulation; the pharmacokinetic considerations for ultrashort peptides; the preclinical pharmacology across cardiac, inflammatory, and aging cell models; the clinical evidence base (absent); sourcing and quality verification; reconstitution and handling; stack interactions and combinations; adverse events and safety signal; and a comparative assessment of five cardioprotective or cardiac-repair peptide candidates (Vesugen, Thymosin beta-4, BPC-157, Cortagen, GHK-Cu) against Cardiogen on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Not assigned. The compound has not received an INN designation from the World Health Organization.

Designated name: Cardiogen. The name is a proprietary designation within the Khavinson peptide bioregulator nomenclature system and is used across the Russian-language and international research literature to identify the AEDR tetrapeptide in its cardiovascular bioregulator application. The name is occasionally transliterated from the Russian as Kardiogen.

One-letter amino acid code: AEDR (Ala-Glu-Asp-Arg).

Three-letter amino acid code: L-Ala-L-Glu-L-Asp-L-Arg.

Full chemical name: L-alanyl-L-glutamyl-L-aspartyl-L-arginine. Alternate designations in the peptide chemistry literature include H-Ala-Glu-Asp-Arg-OH (indicating unprotected N-terminal alpha-amino and C-terminal carboxyl groups).

Chemical Abstracts Service registry number: Not uniquely assigned as a pharmaceutical entity at the time of monograph preparation. The tetrapeptide sequence Ala-Glu-Asp-Arg is a defined sequence within general peptide nomenclature but has not received a unique CAS number in the manner of small-molecule pharmaceuticals. Investigators should reference the compound by its full sequence (H-Ala-Glu-Asp-Arg-OH or AEDR) and by its molecular formula for unambiguous identification.

PubChem Compound Identifier: CID 11583989 (H-Ala-Glu-Asp-Arg-OH) [16].

1.2 Structural class and chemistry

Cardiogen is an ultrashort linear tetrapeptide composed of four alpha-amino acid residues joined by standard alpha-peptide bonds. The structural class places it within the Khavinson ultrashort peptide bioregulator family, a series of synthetic two-to-seven-residue peptides designed as minimal pharmacophore units of tissue-specific polypeptide complexes isolated from mammalian organs by acid-pepsin

hydrolysis and ultrafiltration [3, 4]. Cardiogen is distinguished within this family by the combination of a hydrophobic neutral residue (alanine) at the N-terminus, two acidic residues (glutamic acid and aspartic acid) at positions two and three, and a basic guanidinium-bearing residue (arginine) at the C-terminus. This charge distribution produces a zwitterionic structure at physiological pH with both negative charge centers from the carboxylate side chains and a positive charge center from the arginine guanidinium group.

Molecular formula: C₁₈H₃₁N₇O₉.

Molecular weight: 489.48 g/mol (calculated from the protonated free peptide form).

Isoelectric point: Approximately 4.0 to 4.5 (calculated), reflecting the balance between two acidic residues (glutamic acid pK_a approximately 4.1; aspartic acid pK_a approximately 3.9), the basic arginine residue (pK_a approximately 12.5), and the terminal amino and carboxyl groups. At physiological pH (7.4), the compound carries a net negative charge of approximately minus 1, with the two carboxylate side chains deprotonated, the arginine guanidinium protonated, the N-terminal amino group protonated, and the C-terminal carboxyl deprotonated.

Physical properties: White to off-white lyophilized powder or crystalline solid. Freely soluble in water, phosphate-buffered saline, and aqueous buffers at neutral to slightly alkaline pH. Sparingly soluble in apolar organic solvents. Hygroscopic. Stable as a lyophilized solid at minus 20 degrees Celsius for at least 24 months when protected from moisture.

Stereochemistry: All four residues are in the L-configuration. No chiral inversion is expected during standard solid-phase or solution-phase peptide synthesis. Investigators should confirm L-stereochemistry by chiral amino acid analysis after acid hydrolysis or by comparison of chromatographic retention time to synthetic standards.

1.3 Synthesis

Cardiogen is synthesized by standard solid-phase peptide synthesis (Fmoc chemistry) or by solution-phase peptide synthesis. The solid-phase approach proceeds from an arginine-preloaded resin (Wang resin or 2-chlorotrityl chloride resin with Fmoc-Arg(Pbf)-OH), with sequential coupling of Fmoc-Asp(OtBu)-OH, Fmoc-Glu(OtBu)-OH, and Fmoc-Ala-OH using standard activating reagents (HBTU, HATU,

or DIC/HOBt). Deprotection of the Fmoc group at each step is carried out with 20 percent piperidine in dimethylformamide. Final cleavage from the resin and simultaneous removal of side-chain protecting groups (the Pbf group from arginine and the OtBu groups from glutamic acid and aspartic acid) is accomplished with trifluoroacetic acid in the presence of scavengers (triisopropylsilane, water). The crude peptide is purified by reversed-phase high-performance liquid chromatography (C18 column, water-acetonitrile gradient with 0.1 percent trifluoroacetic acid) to greater than 95 percent purity. Lyophilization of the purified fraction yields the final product as the trifluoroacetate salt or, after counterion exchange, as the acetate salt. The solution-phase synthesis proceeds by sequential condensation of the four amino acids using carbodiimide or active ester chemistry with appropriate side-chain protection. The synthesis is straightforward; the four-residue chain length and the absence of sterically demanding coupling steps result in high coupling efficiencies and overall yields typically exceeding 50 to 65 percent after purification [17].

2. Abstract

Cardiogen (H-Ala-Glu-Asp-Arg-OH; AEDR) is a synthetic tetrapeptide bioregulator developed at the Saint Petersburg Institute of Bioregulation and Gerontology by Vladimir Khavinson and colleagues as the cardiac-specific member of a family of ultrashort peptide bioregulators designed to recapitulate the tissue-specific regulatory activity of organ-derived polypeptide complexes [1, 2, 3]. The compound is classified as the cardiovascular system bioregulator within the Khavinson peptide nomenclature, a designation based on the tissue-specificity demonstrated in organotypic culture experiments in which each tetrapeptide stimulated proliferation selectively in its corresponding tissue type [18].

The compound operates through a multi-level molecular mechanism. At the epigenetic level, Cardiogen and structurally related Khavinson ultrashort peptides have been demonstrated to penetrate cell nuclei and interact directly with double-stranded DNA in gene promoter regions and with histone proteins (H1, H2B, H3, H4), producing chromatin decondensation and altered transcriptional availability of cardiac-specific gene loci [5, 6, 7]. The AEDR sequence was shown to bind fluorescein isothiocyanate-tagged wheat histones in the N-terminal peptide-binding motif regions and to either inhibit or stimulate hydrolysis of bacteriophage lambda DNA by eukaryotic endonucleases WEN1 and WEN2, depending on the methylation status of the DNA substrate [7]. This epigenetic mechanism is proposed as the molecular basis for the tissue-specific cardioprotective activity, wherein the tetrapeptide alters chromatin conformation at cardiac-relevant gene promoters to increase transcriptional accessibility.

At the cellular level, the principal characterized activity of Cardiogen is stimulation of cardiomyocyte proliferation and suppression of cardiomyocyte apoptosis. In organotypic myocardial tissue cultures from young (3-month-old) and aged (24-month-old) rats, Cardiogen at a concentration of 10 to the power of minus 12 molar demonstrated stimulatory effects on proliferation in both age groups, with immunohistochemical analysis revealing decreased p53 protein expression as the probable anti-apoptotic mechanism [9, 10]. In embryonic fibroblast cultures, the tetrapeptide increased expression of cytoplasmic proteins actin, vimentin, and tubulin by up to five-fold and nuclear matrix proteins lamin A and lamin C by up to

2.5-fold relative to untreated control cells [8]. The upregulation of cytoskeletal and nuclear matrix proteins is interpreted as the molecular basis for enhanced cell proliferation, structural integrity, and resistance to apoptosis in cardiac tissue.

In a coronary artery ligation model of experimental myocardial infarction, Cardiogen treatment was reported to produce a threefold reduction in mortality relative to control, with histological evidence of reduced necrotic zones, preserved myocardial glycogen stores, and protection of cellular structures involved in energy production [11, 13]. The cardioprotective effect in this ischemia model is consistent with the anti-apoptotic and cytoprotective mechanisms characterized in cell culture.

A separate investigation examined the tumor-modifying effect of Cardiogen on transplanted M-1 sarcoma in senescent rats. Dose-dependent inhibition of tumor growth was observed, mediated by hemorrhagic necrosis and stimulation of tumor cell apoptosis; the mechanism was attributed to vascular effects on the tumor microenvironment rather than direct cytostatic activity on tumor cells [12].

All published Cardiogen research is preclinical. No human clinical trials of any design have been published in peer-reviewed literature. No pharmacokinetic data from human subjects are available. The compound is not approved for therapeutic use by any Western regulatory authority. It is registered in the Russian Federation as a biologically active additive (a regulatory category analogous to a dietary supplement) and is commercially available there in oral capsule formulations. The entirety of the published preclinical evidence originates from the Khavinson research network at the Saint Petersburg Institute of Bioregulation and Gerontology and affiliated Russian laboratories. No independent replication by any Western research group has been published. Investigators considering Cardiogen for research applications should weight the evidence base accordingly.

3. Discovery and Development History

3.1 Origin

Cardiogen was developed at the Saint Petersburg Institute of Bioregulation and Gerontology (formerly the Military Medical Academy laboratory of bioregulators) under the direction of Vladimir Khavinson as part of a systematic program to identify ultrashort peptide sequences with tissue-specific bioregulatory activity [1, 2]. The Khavinson bioregulatory peptide program began in the late 1970s and early 1980s with the extraction, fractionation, and characterization of polypeptide complexes from mammalian organ extracts (thymus, pineal gland, brain cortex, blood vessels, heart, prostate, pancreas, bronchi, and other organs). These polypeptide complexes, obtained by acid-pepsin hydrolysis and ultrafiltration, were designated "Cytomedins" and demonstrated tissue-specific biological activity in animal models and, subsequently, in clinical applications within the Soviet and Russian healthcare systems [19, 20].

The second phase of the Khavinson program, beginning in the 1990s and continuing through the 2000s, involved identification of the minimal bioactive peptide sequences within the polypeptide complexes. Using iterative fractionation, amino acid analysis, and systematic synthesis of candidate sequences, the Khavinson laboratory identified a series of di-, tri-, and tetrapeptide sequences that retained the tissue specificity of their parent polypeptide complexes. Cardiogen (AEDR) was identified as the cardiac-specific tetrapeptide bioregulator through this process, representing the minimal peptide sequence from cardiac tissue extracts that maintained heart-specific proliferative and cytoprotective activity [3, 4].

3.2 Relationship to the broader Khavinson peptide family

Cardiogen occupies a defined position within a family of structurally related Khavinson tetrapeptides that share the Ala-Glu-Asp tripeptide core at positions one through three but differ at the fourth position, with this single amino acid substitution determining tissue specificity [4]. The principal family members are Cardiogen (Ala-Glu-Asp-Arg; cardiac), Cortagen (Ala-Glu-Asp-Pro; cortical/nervous system), and Epithalon (Ala-Glu-Asp-Gly; pineal/neuroendocrine). The conservation of the

Ala-Glu-Asp core across these tissue-specific bioregulators suggests a common epigenetic regulatory mechanism (DNA and histone binding through the acidic dipeptide motif Glu-Asp) with tissue-targeting specificity conferred by the C-terminal residue. In the case of Cardiogen, the arginine residue at position four is the principal structural determinant of cardiac specificity, though the molecular basis for this specificity (whether it reflects selective DNA sequence recognition, differential histone-binding affinity, or preferential cellular uptake in cardiac tissue) has not been fully elucidated.

3.3 Tissue-specificity demonstration

The tissue specificity of Cardiogen was formally established in the Chalisova et al. (2006) organotypic tissue culture study, in which four tetrapeptide bioregulators (Cardiogen, Bronchogen, Prostamax, and Pancragen) were applied to organotypic tissue cultures of explants from heart, lungs, prostate, and pancreas of young (3-week-old) and aged (18-month-old) rats [18]. Each tetrapeptide stimulated proliferation selectively in only the corresponding tissue type, with Cardiogen producing significant stimulatory effects on myocardial tissue proliferation in both age groups. The effective concentration was 0.05 ng/mL, corresponding to a molar concentration of approximately 10^{-10} to 10^{-11} molar, a range characteristic of the Khavinson ultrashort peptide bioregulators and substantially below conventional pharmacological dose ranges for peptide therapeutics.

3.4 Patent and regulatory status

Cardiogen is covered by Russian patents held by the Saint Petersburg Institute of Bioregulation and Gerontology. The compound is registered in the Russian Federation as a biologically active additive (BAD), a regulatory classification that permits over-the-counter sale but does not constitute pharmaceutical approval. The compound has not been submitted to any Western regulatory authority (FDA, EMA, TGA, PMDA) for pharmaceutical registration. No investigational new drug (IND) application has been filed.

4. Mechanism of Action

Cardiogen operates through a multi-level molecular mechanism that comprises epigenetic regulation of gene expression through peptide-DNA and peptide-histone interactions, downstream modulation of cardiac-specific protein expression, and anti-apoptotic signaling through p53 pathway suppression. The following sections describe each mechanistic level based on the available preclinical evidence.

4.1 Peptide-DNA interaction and epigenetic regulation

The foundational mechanistic hypothesis for Cardiogen and all Khavinson ultra-short peptides is that short peptide sequences (two to seven residues) can penetrate cell nuclei and nucleoli and interact directly with the nucleosome, with histone proteins, and with both single-stranded and double-stranded DNA, producing changes in chromatin conformation that alter gene transcriptional accessibility [5, 6, 7]. This hypothesis, developed systematically across the Khavinson peptide family, rests on several lines of experimental evidence.

First, the AEDR tetrapeptide binds fluorescein isothiocyanate-tagged wheat histones H1, H2B, H3, and H4 in the region of the N-terminal peptide-binding motifs, in common with the related peptides AEDG (Epithalon), KEDW (Pancragen), and others in the family [7]. The histone-binding activity suggests that AEDR can interact with chromatin structural proteins and potentially alter the accessibility of DNA wrapped around nucleosome cores.

Second, AEDR either inhibits or stimulates hydrolysis of bacteriophage lambda DNA by eukaryotic endonucleases WEN1 and WEN2, with the direction of effect depending on the methylation status of the DNA substrate [7]. This finding indicates that the peptide-DNA interaction is sensitive to epigenetic modifications (DNA methylation) and that the functional consequence of the interaction (transcriptional activation or repression) depends on the local epigenetic state of the target gene.

Third, molecular modeling and docking studies across the Khavinson peptide family have demonstrated that ultrashort peptides can fit within the minor and major grooves of double-stranded DNA at specific sequence motifs, with binding affini-

ties and orientations that vary by peptide sequence [6]. While the specific DNA sequence motif recognized by AEDR has not been published in the manner that KEDW has been characterized as binding the ACCT sequence in pancreatic gene promoters, the general mechanism of sequence-specific DNA binding in gene promoter regions is established for the peptide class.

The composite epigenetic mechanism proposes that Cardiogen penetrates cardiac cell nuclei, binds to histone proteins and DNA in the promoter regions of cardiac-specific genes, produces chromatin decondensation (deheterochromatinization) at these loci, and thereby increases the transcriptional availability of genes encoding cytoskeletal proteins, nuclear matrix proteins, and anti-apoptotic factors. In aged cardiac tissue, where progressive heterochromatin condensation restricts access to cardiac gene loci, this mechanism would restore transcriptional programs that are silenced during cardiac aging.

4.2 Cytoskeletal and nuclear matrix protein upregulation

The downstream consequence of the epigenetic mechanism was characterized in the Khavinson, Lin'kova, Polyakova et al. (2012) study of Cardiogen effects on mouse embryonic fibroblast cultures [8]. Treatment with H-Ala-Glu-Asp-Arg-OH produced upregulation of cytoplasmic proteins actin, vimentin, and tubulin by up to five-fold relative to untreated control cells, and upregulation of nuclear matrix proteins lamin A and lamin C by up to 2.5-fold. The cytoskeletal proteins (actin, vimentin, tubulin) form the internal structural framework of cardiomyocytes and are essential for maintaining the contractile architecture that allows heart muscle to generate mechanical force. The nuclear matrix proteins (lamins A and C) are intermediate filament proteins of the nuclear lamina that maintain nuclear structural integrity, regulate chromatin organization, and participate in DNA replication and transcription. The upregulation of both protein classes is consistent with enhanced cell proliferative capacity, improved structural integrity, and resistance to mechanical and ischemic stress in cardiac tissue.

4.3 Anti-apoptotic mechanism

The anti-apoptotic activity of Cardiogen was characterized in the Chalisova et al. (2009) organotypic myocardial tissue culture study [9, 10]. Immunohistochemical analysis of myocardial tissue explants from both young and aged rats treated with Cardiogen at 10^{-12} molar demonstrated decreased expression

of the p53 tumor suppressor protein. The p53 protein is a central regulator of apoptosis in cardiomyocytes; its activation in response to oxidative stress, DNA damage, and ischemic injury drives mitochondrial apoptotic pathway activation and cardiomyocyte death. The reduction of p53 expression by Cardiogen is the proposed molecular mechanism for the anti-apoptotic and cytoprotective activity observed in myocardial tissue cultures and in the coronary artery ligation model. The finding that p53 suppression occurred in both young and aged tissue suggests that the mechanism is operative across the lifespan and is not restricted to the context of age-related cardiac decline.

4.4 Tissue-specific proliferative effect

Cardiogen demonstrates tissue-specific stimulation of proliferation that distinguishes it from a non-specific growth factor. In the Chalisova et al. (2006) organotypic tissue culture study, Cardiogen stimulated proliferation in myocardial tissue but not in lung, prostate, or pancreatic tissue explants [18]. This tissue specificity, observed consistently across the Khavinson tetrapeptide family, is attributed to the C-terminal amino acid residue (arginine in Cardiogen) and to the interaction of the complete tetrapeptide sequence with tissue-specific DNA promoter sequences and histone-binding sites. The effective concentration (0.05 ng/mL; approximately 10 to the power of minus 10 molar) is characteristic of the Khavinson ultrashort peptides and is orders of magnitude below the micromolar concentrations at which most conventional peptide agonists operate, suggesting a catalytic or signaling-amplification mechanism rather than stoichiometric receptor occupancy.

4.5 Vascular and anti-inflammatory mechanisms

The Khavinson, Linkova, and Dyatlova (2023) review of the senescence-associated secretory phenotype (SASP) in cardiovascular system cells characterized Cardiogen (AEDR tetrapeptide) as a regulator of molecules involved in inflammaging and SASP formation in cardiovascular cells [13]. The specific anti-inflammatory mechanisms attributed to Cardiogen include modulation of cytokine expression in aged cardiac and vascular cells, reduction of pro-inflammatory signaling associated with cellular senescence, and regulation of the senescence-associated secretory phenotype that contributes to chronic low-grade inflammation in the aging cardiovascular system. These anti-inflammatory and anti-senescence properties are mechanistically

coherent with the epigenetic gene-regulatory mechanism described above and represent an extension of the Cardiogen activity profile from acute cardioprotection to chronic cardiovascular aging.

5. Pharmacokinetics and Routes of Administration

5.1 General pharmacokinetic considerations for ultrashort peptides

No formal pharmacokinetic study has been published for Cardiogen as the isolated synthetic AEDR tetrapeptide in any species. The pharmacokinetic profile is therefore inferred from the general properties of the ultrashort peptide class and from studies conducted on structurally related Khavinson peptides.

As a linear tetrapeptide with unprotected N-terminal alpha-amino and C-terminal carboxyl groups, Cardiogen is expected to undergo rapid proteolytic degradation by ubiquitous aminopeptidases, carboxypeptidases, and endopeptidases in plasma, gastrointestinal fluid, and tissue interstitium. The plasma half-life of unprotected tetrapeptides of common L-amino acids is generally measured in seconds to minutes in in vitro plasma stability assays and in low single-digit minutes in vivo in rodent studies [14, 15].

5.2 Absorption

Oral absorption of ultrashort peptides, despite their rapid proteolytic degradation, has been proposed to occur through the proton-coupled oligopeptide transporter (POT) family, specifically through the intestinal transporter PEPT1 (SLC15A1) and the renal and neural transporter PEPT2 (SLC15A2) [14, 15]. Molecular modeling studies from the Khavinson laboratory have demonstrated that ultrashort peptides including di- and tripeptide degradation products of Cardiogen are substrates of these active transport carriers, supporting a mechanism for intestinal uptake despite the proteolytic environment of the gastrointestinal lumen. The oral bioavailability of the intact tetrapeptide has not been measured. The biologically active species may include the intact tetrapeptide, its constituent di- and tripeptide fragments (Ala-Glu, Glu-Asp, Asp-Arg, Ala-Glu-Asp, Glu-Asp-Arg), or the free amino acids themselves; the relative contribution of each species to the observed biological activity has not been formally delineated.

5.3 Distribution

No distribution data are available for Cardiogen. Based on the physicochemical properties of the compound (molecular weight 489.48, high aqueous solubility, zwitterionic character, low lipophilicity), the volume of distribution is expected to be moderate, with distribution primarily in the aqueous compartments and limited passive blood-brain barrier penetration. Tissue-specific uptake through active transport mechanisms (PEPT1/PEPT2 and potentially other peptide transporters expressed in cardiac tissue) may contribute to cardiac distribution, consistent with the tissue-specific biological activity observed in preclinical models.

5.4 Metabolism

Cardiogen is expected to be metabolized by the standard proteolytic pathways that degrade all linear peptides. The principal metabolic enzymes include dipeptidyl peptidases, aminopeptidases (particularly aminopeptidase N and aminopeptidase M), carboxypeptidases, and tissue-specific endopeptidases. The metabolic products are the constituent amino acids (L-alanine, L-glutamic acid, L-aspartic acid, L-arginine), which enter the standard amino acid metabolic pools.

5.5 Elimination

Elimination of the intact peptide and its degradation products is expected to proceed through renal excretion of amino acids and small peptide fragments and through incorporation of the constituent amino acids into endogenous metabolic pathways. No renal clearance or biliary excretion data are available.

5.6 Routes of administration

Two principal routes are described in the research and commercial literature:

Oral administration: Cardiogen is commercially available in the Russian Federation as oral capsules containing the tetrapeptide in combination with excipients. The typical dosing described in the commercial literature is one to two capsules daily for 10 to 30 days. No pharmacokinetic validation of oral dosing has been published.

Parenteral administration (research): For in vitro and animal research, Cardiogen is dissolved in physiological saline or phosphate-buffered saline and administered by intraperitoneal or subcutaneous injection. The effective concentrations in cell culture studies range from 10^{-12} to 10^{-7} molar, with the most commonly reported effective concentration being 0.05 ng/mL (approximately 10^{-10} molar) [18].

6. Preclinical Pharmacology

6.1 Organotypic myocardial tissue culture studies

The foundational preclinical evidence for Cardiogen comes from organotypic tissue culture experiments conducted at the Saint Petersburg Institute of Bioregulation and Gerontology and affiliated institutions.

The Chalisova et al. (2006) study examined the tissue-specific effects of four synthetic tetrapeptide bioregulators (Cardiogen, Bronchogen, Prostamax, Pancragen) in organotypic tissue cultures of heart, lung, prostate, and pancreas explants from young (3-week-old) and aged (18-month-old) rats [18]. Cardiogen at 0.05 ng/mL selectively stimulated proliferation in myocardial tissue cultures from both young and aged rats. The stimulatory effect was specific to heart tissue; Cardiogen did not significantly stimulate proliferation in lung, prostate, or pancreatic tissue cultures. This finding established the tissue specificity of the tetrapeptide and confirmed that the single amino acid difference at position four (arginine for Cardiogen versus leucine for Bronchogen, lysine for Prostamax, and tryptophan for Pancragen) was the structural determinant of organ selectivity.

The Chalisova et al. (2009) study extended the myocardial tissue culture work to examine the effects of 20 individual amino acids and Cardiogen on myocardial tissue cultures from young (3-month-old) and aged (24-month-old) rats [9, 10]. Cardiogen at 10 to the power of minus 12 molar demonstrated the greatest stimulatory effect on proliferation in myocardial tissue from both age groups. Immunohistochemical analysis demonstrated decreased p53 protein expression in Cardiogen-treated explants, providing the molecular basis for the anti-apoptotic effect. Cardiogen was more effective than any of the individual amino acids tested, demonstrating that the biological activity requires the intact tetrapeptide sequence and is not attributable to any single constituent amino acid.

6.2 Embryonic fibroblast protein expression study

The Khavinson, Lin'kova, Polyakova et al. (2012) study examined the effect of H-Ala-Glu-Asp-Arg-OH on expression of cytoskeletal and nuclear matrix proteins in mouse embryonic fibroblast cultures [8]. Treatment with the tetrapeptide increased

expression of cytoplasmic proteins actin, vimentin, and tubulin by two-fold to five-fold relative to control, and nuclear matrix proteins lamin A and lamin C by two-fold to 2.5-fold. The study used immunocytochemistry to quantify protein expression levels and demonstrated that the effect was statistically significant across replicate experiments. The authors interpreted the cytoskeletal and nuclear matrix protein upregulation as the mechanistic basis for the previously reported cardioprotective activity, with enhanced structural protein expression supporting cell proliferation, structural integrity, and resistance to apoptosis.

6.3 Coronary artery ligation model

Cardiogen has been evaluated in an experimental coronary artery ligation model of myocardial infarction in rats [11, 13]. The reported findings include a threefold reduction in mortality in treated versus control groups, reduced extent of necrotic zones from ischemic injury, preserved myocardial glycogen stores (an indicator of maintained cellular energy reserves), and histological evidence of protection of mitochondria and other cellular structures involved in oxidative phosphorylation. The experimental details of this study (dose, route, timing relative to ligation, sample size, statistical analysis) are reported in summary form in secondary sources and in the Khavinson, Linkova, and Dyatlova (2023) review; a fully detailed primary report with complete methodology has not been identified in the peer-reviewed English-language literature.

6.4 Tumor-modifying effect on M-1 sarcoma

The Levдик and Knyazkin (2009) study examined the tumor-modifying effect of Cardiogen on transplanted M-1 sarcoma in senescent rats [12]. Cardiogen injection produced dose-dependent inhibition of sarcoma growth. Histological examination revealed that the inhibition was mediated by hemorrhagic necrosis within the tumor and by stimulation of tumor cell apoptosis. The level of apoptosis in tumor cells was higher in all experimental groups receiving Cardiogen than in the control group. The authors concluded that the antitumor effect was not a direct cytostatic action on tumor cells but rather a vascular mechanism, with Cardiogen affecting the tumor vascular network in a manner that produced hemorrhagic necrosis and secondary tumor cell death. This finding is pharmacologically coherent with the vascular regulatory activity attributed to Cardiogen and related Khavinson peptides.

6.5 Limitations of the preclinical evidence

Several methodological limitations constrain interpretation of the preclinical evidence for Cardiogen. All published studies originate from a single research network (the Khavinson group and affiliated Russian institutions). No independent replication by any Western laboratory has been published. Sample sizes in the animal studies are not consistently reported in the available English-language literature. The organotypic tissue culture studies use proliferation indices and immunohistochemical staining as primary endpoints, which are semi-quantitative and subject to observer bias. The coronary artery ligation data are reported in summary form without complete statistical methodology. The tumor-modifying study in senescent rats, while published in a peer-reviewed journal (*Bulletin of Experimental Biology and Medicine*), represents a single study without replication. Investigators should regard the preclinical evidence as hypothesis-generating rather than definitive.

7. Clinical Evidence Base

No human clinical trials of Cardiogen have been published in peer-reviewed literature. No randomized controlled trials, no open-label studies, no case series, and no pharmacokinetic studies in human subjects are available. The compound has not been evaluated in any clinical investigation registered on ClinicalTrials.gov, the EU Clinical Trials Register, or other major clinical trial registries.

The clinical context for Cardiogen derives entirely from the broader Khavinson peptide bioregulator program. The parent program has produced clinical data for other peptide bioregulators, most notably Thymalin (thymic polypeptide complex) and Epithalamin (pineal polypeptide complex), which were evaluated in a 266-patient, 6-to-8-year prospective study that reported reduced mortality and improved functional parameters in elderly patients [20, 21]. These clinical results pertain to the polypeptide complexes rather than to the isolated ultrashort peptides and cannot be directly extrapolated to Cardiogen.

Cardiogen is registered in the Russian Federation as a biologically active additive and is commercially available there in oral capsule formulations. This registration does not constitute evidence of clinical efficacy or safety in the sense of Western pharmaceutical regulatory standards. The regulatory pathway for biologically active additives in Russia does not require the clinical trial evidence that would be required for pharmaceutical approval by the FDA, EMA, or other major Western regulatory authorities.

The absence of any human clinical data represents the most significant limitation in the evidence base for Cardiogen. All claims of efficacy are based on preclinical cell culture and animal model experiments. Investigators considering Cardiogen for any research application involving human subjects should be aware that no human safety or efficacy data exist to inform risk-benefit assessment, dose selection, or adverse event monitoring.

8. Sourcing and Quality Verification

Research-grade Cardiogen is supplied by multiple peptide synthesis vendors internationally (Peptide Sciences, NovoPro Labs, various research peptide suppliers) at greater than 95 percent purity by high-performance liquid chromatography. The compound is supplied as a lyophilized powder of the trifluoroacetate or acetate salt in milligram quantities, typically in 20 mg vials. Pricing is moderate, in the range of \$30 to \$80 per 20 mg vial of research-grade material at the time of monograph preparation.

Commercial-grade Cardiogen is available in the Russian Federation from Peptide Products (the Khavinson laboratory commercial arm) and affiliated distributors as oral capsules and sublingual formulations. These commercial preparations are manufactured under Russian regulatory standards for biologically active additives, which are less stringent than pharmaceutical good manufacturing practice (GMP) standards applicable to drug products.

Investigators should confirm compound identity and purity on every research-grade lot through:

- Reversed-phase high-performance liquid chromatography area-percent purity, ideally greater than 95 percent
- Mass spectrometry confirmation of molecular weight (490.49 plus or minus 0.5 for the protonated species $[M+H]^+$, or 489.48 plus or minus 0.5 for the neutral form)
- Amino acid analysis after acid hydrolysis to confirm the presence of alanine, glutamic acid, aspartic acid, and arginine in equimolar ratios (1:1:1:1)
- Peptide sequencing (Edman degradation or tandem mass spectrometry) to confirm the AEDR sequence order, as sequence isomers (AERD, AEDR, ADRE, etc.) are not distinguishable by molecular weight alone
- Residual solvent analysis and endotoxin testing for parenteral research applications

Third-party analytical laboratories provide independent confirmation of vendor certificates of analysis. Given that the Cardiogen research market is smaller and less established than the markets for mainstream research peptides, lot-to-lot vari-

ability is a plausible concern, and on-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important.

9. Reconstitution and Handling

Cardiogen as the lyophilized trifluoroacetate or acetate salt is freely soluble in water at room temperature. For research applications, the compound is reconstituted as needed in sterile water for injection, bacteriostatic water, normal saline, or phosphate-buffered saline.

Stock solution preparation: A typical stock solution is prepared at 1 to 10 mg/mL in the chosen vehicle. Given the biological activity at picomolar to nanomolar concentrations reported in the preclinical literature, serial dilution from a concentrated stock is required to achieve working concentrations. Investigators should use polypropylene tubes and tips to minimize adsorptive losses, as short peptides can adsorb to glass and polystyrene surfaces.

Storage of solid: Store the lyophilized powder at minus 20 degrees Celsius, protected from moisture, for long-term storage (up to 24 months). Short-term storage at 2 to 8 degrees Celsius is acceptable for periods up to 3 months.

Storage of reconstituted solutions: Aqueous stock solutions should be aliquoted immediately after preparation to avoid repeated freeze-thaw cycles. Aliquots stored at minus 20 degrees Celsius are stable for at least 3 months. Working dilutions in physiological saline or buffer should be used within 24 hours of preparation at room temperature or within 14 days at 2 to 8 degrees Celsius when prepared with bacteriostatic water. Light protection is not required but is reasonable practice.

Sterilization: For parenteral research applications, reconstituted solutions should be filter-sterilized through a 0.22-micrometer polyethersulfone or polyvinylidene fluoride membrane. The low molecular weight of the tetrapeptide (489.48 g/mol) ensures complete passage through sterilizing-grade filters.

10. Stack Interactions and Combinations

Cardiogen does not have characterized drug-drug interactions in the conventional pharmacological sense because no pharmacokinetic or clinical studies have been conducted. The following interaction considerations are based on the known molecular mechanisms and on the general principles of peptide bioregulator pharmacology within the Khavinson system.

Vesugen (KED; Lys-Glu-Asp): Vesugen is the Khavinson vascular endothelial bioregulator. The combination of Cardiogen (cardiac muscle) and Vesugen (vascular endothelium) is the most commonly discussed peptide bioregulator combination for cardiovascular applications in the Khavinson literature. The mechanistic rationale is complementary tissue targeting: Cardiogen acts on cardiomyocytes and cardiac fibroblasts, while Vesugen acts on vascular endothelial cells to normalize endothelin-1 expression, restore connexin expression, and increase sirtuin-1 (SIRT1) expression [22]. No controlled study of the combination has been published.

Epithalon (AEDG; Ala-Glu-Asp-Gly): Epithalon is the pineal bioregulator and telomerase activator. The combination of Cardiogen and Epithalon is discussed in the Khavinson geroprotective literature as a multi-organ aging intervention. The two peptides share the Ala-Glu-Asp core and differ only at position four; no pharmacological interaction between the two tetrapeptides has been characterized.

Cortagen (AEDP; Ala-Glu-Asp-Pro): Cortagen is the cortical bioregulator. The combination of Cardiogen and Cortagen has not been studied. The structural similarity (Ala-Glu-Asp core, differing only at position four: arginine versus proline) raises the theoretical question of whether the two peptides might compete for the same DNA-binding or histone-binding sites; no data address this question.

Conventional cardiovascular medications: No interaction data are available. Given that Cardiogen operates through an epigenetic gene-regulatory mechanism rather than through conventional receptor pharmacology, the likelihood of direct pharmacokinetic or pharmacodynamic interaction with standard cardiovascular

drugs (beta-blockers, ACE inhibitors, statins, antiplatelet agents, anticoagulants) is considered low. However, the absence of any human pharmacological data means that no interaction can be formally excluded.

Other cardioprotective peptides (BPC-157, Thymosin beta-4): The combination of Cardiogen with BPC-157 or Thymosin beta-4 for cardiac repair is plausible from a mechanistic perspective (Cardiogen acting through epigenetic gene regulation, BPC-157 through VEGF/NO-mediated angiogenesis, Thymosin beta-4 through actin-mediated cell migration and cardiac progenitor cell activation) but has not been studied in any published model.

11. Adverse Events and Safety Signal

11.1 Preclinical safety

No formal toxicology studies of Cardiogen have been published in the peer-reviewed literature. The available preclinical studies (organotypic tissue culture, embryonic fibroblast culture, coronary artery ligation model, M-1 sarcoma model) do not report adverse events or toxicity findings. The Khavinson ultrashort peptide bioregulator program generally reports favorable safety profiles for the peptide class, attributing the low toxicity to the fact that the compounds are composed of standard L-amino acids and are degraded to their constituent amino acids by normal proteolytic pathways [1, 2].

11.2 Clinical safety

No human safety data are available. No adverse events have been reported in clinical trials because no clinical trials have been conducted. Anecdotal safety information from commercial use in the Russian Federation is not captured in the peer-reviewed literature and cannot be evaluated for reliability.

11.3 Theoretical safety considerations

Several theoretical safety considerations apply to Cardiogen based on its mechanism of action and structural properties:

Proteolytic degradation products: The degradation of Cardiogen produces L-alanine, L-glutamic acid, L-aspartic acid, and L-arginine, all of which are standard dietary amino acids present in substantially higher quantities in normal protein digestion. Toxicity from the degradation products at the picomolar to nanomolar effective concentrations is implausible.

Epigenetic effects: The proposed mechanism of chromatin decondensation and gene expression modulation raises theoretical concerns about unintended gene activation. The tissue specificity demonstrated in organotypic culture experiments provides some reassurance that the effect is selective rather than global, but the

specificity has been demonstrated only in the limited organ panel tested (heart, lung, prostate, pancreas) and has not been evaluated across the full range of human tissues.

Anti-apoptotic activity: The suppression of p53 expression, while cardioprotective in the context of ischemic injury, raises theoretical concerns about impaired tumor surveillance. The Levdik and Knyazkin (2009) study partially addresses this concern by demonstrating that Cardiogen increased tumor cell apoptosis in the M-1 sarcoma model through a vascular mechanism [12], suggesting that the anti-apoptotic effect may be tissue-context-dependent rather than a generalized suppression of p53 function. However, the interaction between the anti-apoptotic and potential pro-tumorigenic effects has not been systematically evaluated.

Immunogenicity: As a tetrapeptide of standard L-amino acids with a molecular weight of 489.48 g/mol, Cardiogen is below the conventional threshold for immunogenicity (generally considered to require molecular weights above approximately 1000 to 3000 g/mol for T-cell-dependent immune responses). The risk of antibody formation or hypersensitivity is considered negligible.

11.4 Pregnancy and lactation

No reproductive toxicology, teratogenicity, or lactation data are available for Cardiogen. The compound should be considered as having an unknown safety profile in pregnancy and lactation.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five cardioprotective, cardiac-repair, or cardiovascular peptide candidates against Cardiogen, evaluated against five competency standards: novelty, effect size, promising potential (current development state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five candidates were selected as the principal peptide compounds with substantive preclinical or clinical evidence for cardiac or cardiovascular applications, with priority on those that occupy adjacent pharmacological or mechanistic space to Cardiogen.

12.1 Vesugen (KED; Lys-Glu-Asp)

Mechanism: Synthetic tripeptide bioregulator of the Khavinson family targeting vascular endothelial cells. Proposed mechanism is epigenetic regulation of vascular gene expression through peptide-DNA and peptide-histone interactions, with downstream normalization of endothelin-1, connexin, and SIRT1 expression in endothelial cells [22].

Development trajectory: Developed at the same institution as Cardiogen (Saint Petersburg Institute of Bioregulation and Gerontology). Registered in Russia as a biologically active additive for vascular health applications. No clinical trials published. Preclinical evidence includes endothelial cell culture studies demonstrating normalization of endothelin-1 expression, restoration of connexin expression, and increased SIRT1 expression. The compound is positioned as the vascular endothelial complement to Cardiogen's cardiomyocyte activity.

Novelty: Low. The mechanism is the standard Khavinson ultrashort peptide epigenetic regulation model applied to vascular endothelial tissue, differing from Cardiogen principally in the target cell type.

Effect size: Preclinical only. Cell culture data demonstrate modulation of endothelial gene expression markers. No quantitative comparison of effect sizes between Vesugen and Cardiogen is available.

Promising potential: Low. No clinical development. No regulatory submissions outside Russia. Evidence base is comparable in size and quality to Cardiogen.

Side-effect profile: No safety data. No human exposure data. Theoretical safety profile is similar to Cardiogen (standard L-amino acid composition, expected rapid proteolytic degradation).

Overall validation: Low. Preclinical evidence from a single research group without independent replication.

Comparison to Cardiogen: Vesugen and Cardiogen occupy complementary positions within the Khavinson cardiovascular peptide bioregulator system, targeting vascular endothelium and cardiac muscle respectively. Neither compound has a stronger evidence base than the other. The combination is the most commonly discussed but least studied pairing in the Khavinson cardiovascular portfolio.

12.2 Thymosin beta-4 (TB-500)

Mechanism: Endogenous 43-amino-acid peptide that is the principal actin-sequestering peptide in mammalian cells. Promotes cell migration through actin polymerization regulation, activates cardiac progenitor cells (epicardium-derived cells), promotes angiogenesis, and activates the ILK-Akt survival signaling pathway. Demonstrated to promote cardiomyocyte survival and improve cardiac function after coronary artery ligation in mice [23, 24].

Development trajectory: Substantially more advanced than Cardiogen. Phase I through Phase III clinical trials have been completed for cardiac applications (RegeneRx Biopharmaceuticals). The compound has been studied in human subjects for cardiac repair following myocardial infarction. Clinical trial data are published in peer-reviewed journals. Preclinical evidence is extensive and includes studies from multiple independent research groups internationally.

Novelty: Moderate. The actin-sequestering mechanism for cardiac repair is well established and the concept of activating endogenous cardiac progenitor cells through Thymosin beta-4 was a significant scientific advance when first demonstrated [23].

Effect size: Substantial in preclinical cardiac models. Demonstrated improvement in cardiac function, reduction in infarct size, and activation of cardiac progenitor cells in multiple independent studies. Clinical effect sizes from Phase II/III

trials are moderate.

Promising potential: Moderate to high. Active clinical development program. Published human safety and efficacy data. Regulatory submissions in progress or completed.

Side-effect profile: Clinical safety data available from multiple human trials. Generally well tolerated. No dose-limiting toxicities reported at studied doses.

Overall validation: High. Extensive preclinical evidence from multiple independent laboratories. Published clinical trial data. The most thoroughly validated cardiac-repair peptide in the current pipeline.

Comparison to Cardiogen: Thymosin beta-4 is superior to Cardiogen on every validation standard. It has a defined molecular mechanism through actin biology, extensive independent preclinical replication, and published clinical trial data. The comparison highlights the principal limitation of Cardiogen: the absence of independent replication and clinical data. The two peptides operate through entirely different mechanisms (epigenetic gene regulation for Cardiogen versus actin-mediated cell migration and progenitor activation for Thymosin beta-4) and are not pharmacologically redundant.

12.3 BPC-157 (Body Protection Compound-157)

Mechanism: Synthetic 15-amino-acid peptide fragment derived from a gastric protein. Promotes angiogenesis through VEGF and NO pathways, stabilizes endothelial barriers, modulates inflammatory cytokines, and demonstrates cytoprotective effects across multiple tissue types including cardiac tissue [25, 26].

Development trajectory: Extensively studied preclinically with over 100 published studies across multiple organ systems. Clinical evidence is limited; a Phase II trial for inflammatory bowel disease has been conducted. Cardiac applications are supported by preclinical studies demonstrating cardioprotective effects in ischemia-reperfusion models and models of heart failure. No cardiac-specific clinical trials have been conducted.

Novelty: Low to moderate. The VEGF/NO angiogenesis mechanism is well characterized. The breadth of tissue activity (gut, musculoskeletal, cardiac, neural) reduces specificity as a cardiac agent.

Effect size: Substantial in preclinical models across multiple tissue types. Cardiac-specific effect sizes are moderate and derive from a smaller number of studies than the musculoskeletal or gastrointestinal evidence base.

Promising potential: Moderate. Active research interest. Wide commercial availability. Clinical development for cardiac applications specifically is limited.

Side-effect profile: Preclinical safety data suggest favorable tolerability. Limited human safety data from clinical trials for non-cardiac indications. No serious adverse events reported.

Overall validation: Moderate. Extensive preclinical evidence, though primarily from a limited number of research groups for the cardiac applications. Clinical validation is emerging for non-cardiac indications.

Comparison to Cardiogen: BPC-157 has a substantially larger preclinical evidence base than Cardiogen, though neither has cardiac-specific clinical data. BPC-157 operates through a different mechanism (VEGF/NO angiogenesis) and lacks the cardiac tissue specificity that Cardiogen demonstrates in organotypic culture. The broader tissue activity of BPC-157 is both an advantage (wider applicability) and a limitation (less cardiac specificity).

12.4 Cortagen (AEDP; Ala-Glu-Asp-Pro)

Mechanism: Synthetic tetrapeptide bioregulator of the Khavinson family targeting cortical and nervous system tissue. Shares the Ala-Glu-Asp tripeptide core with Cardiogen but has proline rather than arginine at position four. Proposed mechanism is epigenetic regulation of neural gene expression through the same peptide-DNA and peptide-histone interaction framework as Cardiogen [4, 27].

Development trajectory: Developed at the same institution as Cardiogen. The preclinical evidence base is comparable in size to Cardiogen, with studies demonstrating tissue-specific effects on neural tissue cultures and gene expression modulation in cortical cells. A gene expression profiling study reported that Cortagen modulated cardiac gene expression in addition to its primary cortical activity, raising questions about the absolute tissue specificity of the Khavinson tetrapeptide system [27]. Registered in Russia as a biologically active additive. No clinical trials published.

Novelty: Low. Same mechanistic framework as Cardiogen, differing only in the tissue-targeting residue.

Effect size: Preclinical only. Comparable to Cardiogen in magnitude and quality of evidence.

Promising potential: Low. No clinical development. Evidence base from a single research group.

Side-effect profile: No safety data. Theoretical profile identical to Cardiogen.

Overall validation: Low. Preclinical evidence from a single research group without independent replication.

Comparison to Cardiogen: Cortagen is the most structurally similar compound to Cardiogen in this comparator panel, differing by a single amino acid at position four (Pro versus Arg). The finding that Cortagen also modulates cardiac gene expression, despite being designated as the cortical bioregulator, raises questions about the precision of tissue targeting within the Khavinson tetrapeptide system and suggests that the tissue specificity may be relative rather than absolute.

12.5 GHK-Cu (Copper tripeptide; Gly-His-Lys copper complex)

Mechanism: Endogenous tripeptide-copper complex with tissue-repair, anti-inflammatory, and gene-expression-modulatory activity. Demonstrated to modulate expression of approximately 4,000 human genes, including genes involved in tissue remodeling, anti-inflammatory signaling, and anti-fibrotic pathways. Promotes wound healing, collagen synthesis, and tissue regeneration across multiple organ systems including skin, lung, and bone [28, 29].

Development trajectory: GHK-Cu has been studied for over four decades since its discovery by Loren Pickart. Extensive preclinical evidence from multiple independent research groups. Clinical use in dermatological applications (wound healing, anti-aging skin care) is well established. Cardiac applications are supported by gene expression studies demonstrating modulation of cardiac-relevant gene networks, but dedicated cardiac preclinical or clinical studies are limited.

Novelty: Moderate. The copper-dependent gene-expression-modulatory mechanism is distinct from the Khavinson peptide-DNA interaction model and from the growth factor and cytokine pathways of the other comparators.

Effect size: Substantial for dermatological and wound-healing applications. Cardiac-specific effect data are limited to gene expression modulation studies.

Promising potential: Moderate. Well-established commercial and clinical use in dermatology. Cardiac-specific development is not a current priority.

Side-effect profile: Clinical safety data available from dermatological use. Generally well tolerated. The copper component introduces considerations around copper toxicity at high doses but is not a concern at the microgram to milligram doses used in practice.

Overall validation: Moderate to high for dermatological applications. Low for cardiac-specific applications.

Comparison to Cardiogen: GHK-Cu and Cardiogen share the conceptual approach of modulating gene expression for tissue repair, but through different mechanisms (copper-mediated gene modulation versus peptide-DNA interaction). GHK-Cu has a substantially larger and more independently replicated evidence base overall, but the cardiac-specific evidence for both compounds is limited. GHK-Cu has the advantage of established clinical use in a non-cardiac indication, providing a safety foundation that Cardiogen lacks.

12.6 Comparator summary

The five compounds represent a spectrum of validation levels and mechanistic approaches for cardiac or cardiovascular peptide interventions. **Thymosin beta-4** is the clear leader in validation, with extensive independent preclinical evidence, published clinical trial data, and a defined mechanism through actin biology and cardiac progenitor cell activation. **BPC-157** and **GHK-Cu** occupy intermediate positions, with substantial preclinical evidence bases (though primarily for non-cardiac applications) and varying degrees of clinical use. **Vesugen** and **Cortagen** are structurally and mechanistically related to Cardiogen and share the same evidence limitations: single-source preclinical data without independent replication or clinical validation.

Cardiogen occupies the lowest validation tier within this comparator panel, distinguished by its cardiac tissue specificity (demonstrated in organotypic culture) and by the coherent multi-level mechanism (epigenetic regulation, cytoskeletal protein upregulation, p53 suppression) but constrained by the absence of independent replication, the absence of human clinical data, and the reliance on a single research network for all published evidence. The principal value of Cardiogen in the current research landscape is as a molecular tool for investigating peptide-mediated epigenetic regulation of cardiac gene expression, a mechanistic concept that is theoretically distinct from the conventional growth factor, cytokine, and receptor-agonist approaches represented by the other comparators.

Investigators considering Cardiogen for cardiac research should calibrate expectations against the validation hierarchy established by this comparative assessment. The compound offers a unique mechanistic hypothesis (ultrashort peptide epigenetic regulation of cardiac genes at picomolar concentrations) that has not been independently validated, and the clinical translation pathway requires substantially more evidence than is currently available.

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This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. All published evidence for Cardiogen is preclinical and originates from a single research network (the Khavinson group at the Saint Petersburg Institute of Bioregulation and Gerontology). No independent replication by Western laboratories and no human clinical trials have been published. Investigators should weight the evidence accordingly and should obtain analytical confirmation of identity and purity on every lot of research-grade material.