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Lipid and vascular biomarker responses to sulfated chitosan in rabbits with experimental hypercholesterolemia

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Abstract. Background: Sulfation modifies the biological properties of chitosan and may produce compounds with activity relevant to vascular disease. The efficacy of an individual preparation requires evaluation independently of findings for other chitosan derivatives. Objective: To describe the lipid and circulating vascular biomarker responses associated with a locally synthesized sulfated chitosan preparation, designated sulfoparin, in cholesterol-exposed rabbits. Methods: Aggregate endpoint data were extracted from a dissertation reporting 46 Chinchilla rabbits allocated to an intact group (n = 6) and five cholesterol-exposed groups (n = 8 each). After two months of cholesterol exposure, treatment continued for one month alongside cholesterol administration. Groups received no active treatment, a fibrate comparator reported as “hemfibrazil” at 100 mg/kg, heparin at 15 IU/kg, or sulfated chitosan at reported doses of 25 or 50 μg/kg. Treatment routes and the unusually low chitosan dose units require verification. Results: Compared descriptively with untreated hypercholesterolemia, sulfated chitosan at the two reported doses was

associated with 53.9% and 55.4% lower total cholesterol, 71.7% and 74.4% lower LDL cholesterol, and 73.1% and 78.3% lower C-reactive protein. NOx was 12.29 and 17.86 µmol/L, respectively, compared with 7.89 µmol/L in untreated animals and 16.68 µmol/L in intact animals. Homocysteine remained numerically above the intact reference at both doses. No new inferential statistics were calculated. Conclusions: The source tables describe substantial lipid and biomarker differences associated with sulfated chitosan treatment. These findings support confirmatory investigation but do not establish clinical efficacy, equivalence to heparin, functional endothelial recovery or an optimal dose. Verification of the compound, dosing records, assays and raw data is essential.

Keywords: sulfated chitosan; sulfoparin; hypercholesterolemia; rabbit; lipoproteins; vascular biomarkers

Introduction

Chitosan derivatives are chemically heterogeneous materials whose biological properties depend on molecular size, substitution and preparation conditions. Sulfation can confer anticoagulant activity, but activity cannot be assumed to be identical across products. Experimental work by Vongchan and colleagues demonstrated anticoagulant activity in a characterized sulfated chitosan [1]. Suwan and colleagues subsequently investigated molecular-weight-dependent interactions involving heparin cofactor II in papain-treated chitosan polysulfates [2]. These studies support a rationale for investigating sulfated derivatives while emphasizing the importance of chemical characterization.

The dissertation underlying the present report describes sulfoparin, a sulfated chitosan preparation produced in Uzbekistan from silkworm-derived material [3]. Its rabbit experiments compared lipid and vascular biomarker measurements after treatment with the investigational compound, a fibrate comparator and heparin. The experimental question is relevant because lipid changes and vascular responses may diverge: a favorable lipid panel does not necessarily establish recovery of endothelial function.

Direct measurements of vascular oxidative stress and endothelium-dependent relaxation in cholesterol-fed rabbits have demonstrated how functional vascular responses can be studied [4]. The present source instead emphasizes circulating biochemical endpoints. Accordingly, the objective of this manuscript is limited to describing treatment-associated differences in serum lipids, inflammatory biomarkers and nitric oxide related measurements. It does not treat those endpoints as proof of anticoagulant efficacy, plaque regression or clinical benefit.

Materials and methods

Experimental design - The source describes 46 Chinchilla rabbits, with six intact controls and five experimental groups of eight animals each. Although one sentence refers to five groups, the six listed groups and their sample sizes sum to 46. Cholesterol dissolved in sunflower oil was administered intragastrically at 0.2 g/kg/day for three months. Treatment began after two months and continued for one month during ongoing cholesterol exposure [3].

The six groups were intact reference animals, untreated hypercholesterolemic animals, hypercholesterolemic animals receiving the fibrate comparator at 100 mg/kg, heparin at 15 IU/kg, and sulfated chitosan at 25 or 50 µg/kg. The fibrate name appears as “hemfibrazil” and likely refers to gemfibrozil, but its identity cannot be established from spelling alone. It is therefore designated the fibrate comparator throughout this draft.

The intact group received vegetable oil at 0.5 mL/kg. The disease-control label contains “N2O,” which may represent a transcription error; no gas exposure is inferred. The route,

frequency and vehicle for each active treatment require confirmation. Neither random allocation nor blinded outcome assessment is adequately documented, and no prospective sample-size calculation is available.

Investigational material - Sulfoparin was attributed to the Institute of Polymer Chemistry and Physics of the Academy of Sciences of Uzbekistan, with synthesis associated with the group of S. Sh. Rashidova and R. Yu. Milusheva. The source describes silkworm-derived chitosan and refers to standardization by deacetylation and sulfate content. It does not provide batch-specific molecular weight, degree and position of sulfation, purity, residual reagents, endotoxin results or analytical spectra sufficient to establish equivalence to other published derivatives.

The reported 25 and 50 μg/kg efficacy doses are retained exactly as presented in the rabbit methods and tables. They must not be silently converted to mg/kg. Because the toxicology section uses different dose ranges and contains internal discrepancies, no cross-species exposure margin is calculated from these nominal doses.

Laboratory measurements - Serum was obtained after a 12-hour fast from ear-vein blood. Lipid fractions and CRP were assessed using a Human biochemical platform, and endothelin-1 was measured by an immunoassay. NOx was assessed using a Griess-based method; reported nitrate reductase activity was measured using a separate incubation procedure. These assays are described in the dissertation, but kit identifiers, rabbit validation, homocysteine methods and complete sample handling information are missing [3].

The source's endothelin-1 and homocysteine units remain unresolved. Their original table units are explicitly flagged rather than interpreted against clinical thresholds. The biochemical endpoints are treated as circulating measurements and not as direct tests of endothelial function.

Data handling and statistical interpretation - Source Tables 4.1–4.3 were used as the numerical basis for this report. The printed ± quantities are reproduced as source-reported dispersion pending confirmation of whether they are standard errors or standard deviations. Percentage reductions versus untreated hypercholesterolemia were calculated as 100 × (untreated mean − treated mean)/untreated mean. Percentage increases were calculated with the numerator reversed. These are derived descriptive contrasts between group means.

The source reports pairwise significance tests, but raw observations, the exact analysis plan and adjustment for multiple comparisons are unavailable. This draft therefore reports neither newly calculated P values nor independently verified superiority, equivalence or noninferiority claims. Confirmation of treatment effects requires reanalysis with animal-level data, including baseline comparability and the original allocation structure. ARRIVE 2.0 provides the reporting framework for completing these details [5].

Ethical statement - The supplied source does not provide a verifiable ethics committee name, approval identifier or approval date. These must be supplied from original records, together with welfare procedures, humane endpoints and the animals' disposition. No retrospective approval or compliance statement has been invented.

Results

Lipid responses - Untreated hypercholesterolemic rabbits had total cholesterol of 9.19 mmol/L and LDL cholesterol of 7.49 mmol/L at the final assessment (Table 1). In the sulfated chitosan groups, total cholesterol was 4.24 and 4.10 mmol/L at the reported 25 and 50 μg/kg doses, respectively. These values were 53.9% and 55.4% below the untreated mean. LDL cholesterol was 2.12 and 1.92 mmol/L, corresponding to descriptive reductions of 71.7% and 74.4%.

HDL cholesterol was 1.82 and 1.92 mmol/L in the two chitosan groups, compared with 1.02 mmol/L in untreated animals. The fibrate comparator and heparin groups had total cholesterol

values of 5.51 and 4.21 mmol/L, respectively. Thus, several chitosan group means were closer to the intact reference than those in the fibrate group. This descriptive ordering does not establish comparative efficacy in the absence of a verified analysis.

Table 1 Lipid measurements after one month of treatment

Group	Total cholesterol	VLDL cholesterol	LDL cholesterol	HDL cholesterol
Intact	4.00 ± 0.08	0.315 ± 0.008	2.05 ± 0.09	1.64 ± 0.05
Untreated HC	9.19 ± 0.16	0.686 ± 0.028	7.49 ± 0.24	1.02 ± 0.13
Fibrate	5.51 ± 0.09	0.496 ± 0.015	3.63 ± 0.06	1.38 ± 0.07
Heparin	4.21 ± 0.22	0.188 ± 0.009	2.34 ± 0.24	1.68 ± 0.11
SC 25†	4.24 ± 0.08	0.305 ± 0.007	2.12 ± 0.08	1.82 ± 0.03
SC 50†	4.10 ± 0.07	0.229 ± 0.002	1.92 ± 0.04	1.92 ± 0.04

Source Table 4.1. All concentrations are mmol/L. HC, hypercholesterolemia; SC, sulfated chitosan. †Reported dose in µg/kg, pending verification. Fibrate: source "hemfibrazil," 100 mg/kg; heparin: 15 IU/kg. Planned n = 6 intact and n = 8 per other group; endpoint completeness is unverified. Values are means ± source-reported dispersion.

Inflammatory and vascular biomarker responses - CRP was 21.88 µg/mL in untreated animals and 5.88 and 4.75 µg/mL in the two sulfated chitosan groups (Table 2). These means represent descriptive reductions of 73.1% and 78.3%. The intact reference was 5.17 µg/mL. The source narrative gives a conflicting fold reduction for the higher dose; the percentages here were recalculated directly from the displayed table means.

Endothelin-1 was numerically lower in all active-treatment groups than in untreated animals. Homocysteine also decreased, but its means of 5.77 and 4.58 in the chitosan groups remained above the intact value of 3.46 on the source's concentration scale. This pattern argues against describing every measured disturbance as completely normalized.

Table 2 Inflammatory and vascular biomarkers after treatment

Group	CRP µg/mL	Endothelin 1	Homocysteine
Intact	5.17 ± 0.40	0.291 ± 0.010	3.46 ± 0.25
Untreated HC	21.88 ± 0.64	0.772 ± 0.060	9.99 ± 0.17
Fibrate	15.00 ± 1.87	0.328 ± 0.016	6.96 ± 0.32

Group	CRP µg/mL	Endothelin 1	Homocysteine
Heparin	5.63 ± 0.18	0.269 ± 0.015	5.09 ± 0.17
SC 25	5.88 ± 0.44	0.311 ± 0.018	5.77 ± 0.28
SC 50	4.75 ± 0.45	0.253 ± 0.016	4.58 ± 0.23

Source Table 4.2. Group and dose abbreviations are as in Table 1. †The source labels both analytes in pg/mL; units remain unverified. Values are means ± source-reported dispersion. Source pairwise P values have not been independently validated and are not reproduced.

Nitric oxide related responses - NOx was 7.89 µmol/L in untreated hypercholesterolemia, 9.33 µmol/L with the fibrate comparator and 17.83 µmol/L with heparin (Table 3). Sulfated chitosan groups had means of 12.29 and 17.86 µmol/L. The lower-dose mean was 26.3% below the intact reference of 16.68 µmol/L, whereas the higher-dose mean was 7.1% above it. The latter should not be described as identical to the intact value.

Reported nitrate reductase activity was 4.78 µmol/min/mg protein in untreated animals and 2.91 and 2.33 in the chitosan groups. The corresponding intact mean was 2.72. The chitosan dose ordering was consistent across several endpoints, but a formal dose-response relationship was not estimated.

Table 3 Nitric oxide related measurements after treatment

Group	NOx µmol/L	Reported NR activity†
Intact	16.68 ± 0.28	2.72 ± 0.19
Untreated HC	7.89 ± 0.31	4.78 ± 0.14
Fibrate	9.33 ± 0.15	3.99 ± 0.29
Heparin	17.83 ± 0.18	2.81 ± 0.18
SC 25	12.29 ± 0.50	2.91 ± 0.13
SC 50	17.86 ± 0.11	2.33 ± 0.14

Source Table 4.3, caption n = 6–8. †NR activity is reported in µmol/min/mg protein. Group abbreviations are as in Table 1. Values are means ± source-reported dispersion. NOx does not independently quantify endothelial nitric oxide synthesis.

Discussion

The extracted results show a broad biochemical response associated with sulfated chitosan treatment during continuing cholesterol exposure. Total and LDL cholesterol were substantially lower than in the untreated group, while CRP and endothelin-1 were also lower and NOx was higher. The combination warrants investigation because it suggests that the response was not confined to one lipid fraction. Nevertheless, each endpoint remains a surrogate measurement within an incompletely documented animal experiment.

Several mechanisms could potentially explain the pattern, including altered intestinal lipid handling, changes in lipoprotein metabolism or secondary effects of reduced lipid exposure. The

present experiment cannot distinguish them. The route of treatment and systemic exposure are not adequately specified; therefore, an intestinal mechanism cannot be assumed, and a direct endothelial mechanism cannot be inferred. The source's proposed effects on lecithin–cholesterol acyltransferase, prostacyclin and cytokine synthesis were not directly measured in the tables used here.

Published sulfated-chitosan experiments support the importance of formulation-specific pharmacology. Vongchan and colleagues investigated a chemically characterized derivative [1], while Suwan and colleagues linked anticoagulant behavior to molecular weight and interaction with heparin cofactor II [2]. These observations justify documenting the present material precisely. They do not establish that sulfoparin has the same binding profile, anticoagulant potency or bioavailability. Coagulation assays, bleeding outcomes and pharmacokinetics would be necessary before attributing a heparin-like therapeutic profile to this preparation.

The comparison with heparin requires similar restraint. Heparin was included as a pharmacological comparator in the source experiment, but the design was not an equivalence or noninferiority study. Numerical proximity to a heparin group mean is insufficient to demonstrate comparable efficacy or safety. Moreover, several endpoints differed in direction or magnitude between groups, and there are no verified treatment-group baseline measurements or exposure data.

NOx illustrates why selecting an optimal dose from this dataset would be premature. The higher reported dose yielded a mean near the heparin value, while the lower dose left a substantial numerical deficit relative to intact animals. Conversely, increasing NOx beyond a reference mean cannot itself establish greater benefit. A dose-selection programme should integrate exposure, functional vascular measurements and adverse effects, rather than choosing a dose from isolated biochemical normalization.

The study also cannot separate a direct anti-inflammatory action from a downstream consequence of lower lipids. Establishing mediation would require appropriately timed measurements, individual-level modeling and preferably independent manipulation of candidate pathways. Functional confirmation should include arterial reactivity or another validated vascular endpoint; direct vascular approaches in earlier rabbit studies provide useful methodological context [4].

Important limitations include uncertain dose units, incompletely identified comparator medication, missing treatment routes, limited chemical characterization and unresolved biomarker units. Some lipid fractions do not reconcile exactly with total cholesterol, and treatment-table narrative statements occasionally conflict with the table values. The sample-size description is internally inconsistent even though the enumerated group sizes sum correctly. These issues limit reproducibility and require source-record reconciliation rather than editorial correction by assumption.

The work is part of the same rabbit programme as the untreated time-course analysis. Shared controls must not be presented as an independent replication. The dissertation also reports prior publications, so novelty and overlap must be assessed against the actual published articles before submission. Editorial disclosure of related papers is necessary when analyses derive from the same dataset [6].

Conclusions

The dissertation tables describe favorable lipid and circulating biomarker differences associated with sulfated chitosan in cholesterol-exposed rabbits. The results provide a rationale for a chemically characterized, rigorously reported confirmatory study. They do not yet establish an

optimal dose, clinical applicability, plaque regression, anticoagulant efficacy or equivalence to an active comparator.

Declarations

Ethics approval: [Original committee, approval number, date and animal-welfare documentation required.]

Funding, competing interests and author contributions: [To be completed and approved by the actual investigators.]

Data availability: Aggregate source tables were available for drafting. The original investigators must provide a final statement and specify access to raw measurements, dosing records and material characterization.

Prior dissemination: Dissertation-derived analysis with shared rabbit controls and possible overlap with previously published articles and abstracts; full disclosure remains necessary.

Writing assistance: AI-assisted English drafting and document preparation were used. Authors must verify the content and adapt disclosure to the selected journal's policy.

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