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Clinical Investigations

Cholesterol Crystals Cause Mechanical Damage to Biological Membranes: A Proposed Mechanism of Plaque Rupture and Erosion Leading to Arterial Thrombosis

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Summary

Background: Plaque rupture and/or erosion is the leading cause of cardiovascular events; however, the process is not well understood. Although certain morphologic characteristics have been associated with ruptured plaques, these observations are of static histological images and not of the dynamics of plaque rupture. To elucidate the process of plaque rupture, we investigated the transformation of cholesterol from liquid to solid crystal to determine whether growing crystals are capable of injuring the plaque cap.

Hypothesis: We hypothesized that during cholesterol crystallization the spatial configuration rapidly changes, causing forceful expansion of sharp-edged crystals that can damage the plaque cap.

Methods: Two experiments were performed in vitro: first, cholesterol powder was melted in graduated cylinders and allowed to crystallize at room temperature. Volume changes from liquid to solid state were measured and timed. Second, thin biological membranes (20–40 μm) were put in the path of growing crystals to determine damage during crystallization.

Results: As cholesterol crystallized, the peak volume increased rapidly by up to 45% over 3 min and sharp-tipped crystals cut through and tore membranes. The amount of cholesterol and peak level of crystal growth correlated directly ($r = 0.98$; $p < 0.01$), as did the amount of cholesterol and rate of crystal growth ($r = 0.99$; $p < 0.01$).

Conclusions: These observations suggest that crystallization of supersaturated cholesterol in atherosclerotic plaques can induce cap rupture and/or erosion. This novel insight may help in the development of therapeutic strategies that can alter cholesterol crystallization and prevent acute cardiovascular events.

Key words: cholesterol crystals, plaque rupture, myocardial infarction

Introduction

Plaque rupture and/or erosion is recognized as the primary cause of arterial thrombosis (Fig. 1).[1–4] The major characteristics of ruptured plaques include a large lipid pool, a thin fibrous cap, and a low number of smooth muscle cells.[5] Furthermore, inflammation consisting of macrophages and lymphocytes is frequently present at the sites of plaque rupture, typically at plaque edges where the fibrous cap inserts into the arterial wall.[6–8] Despite the fact that these seem to be recurrent observations on histology, they represent the endstage of the rupture process and do not explain the sequence of events leading to plaque rupture. This study proposes a novel mechanism based on physical alterations within the plaque that may lead to rupture and/or erosion. These changes are related to the alterations in the physical state of the plaque content, primarily cholesterol.

Cholesterol is an organic chemical that has specific crystal characteristics. The physical state of cholesterol is influenced by cholesterol concentration, surrounding medium, pH, temperature, and pressure.[9–12] As for many chemicals, the liquid state has a different spatial distribution than the crystalline solid state, as water is an example of volume expansion after freezing.[13] Cholesterol crystals are frequently seen in athero-

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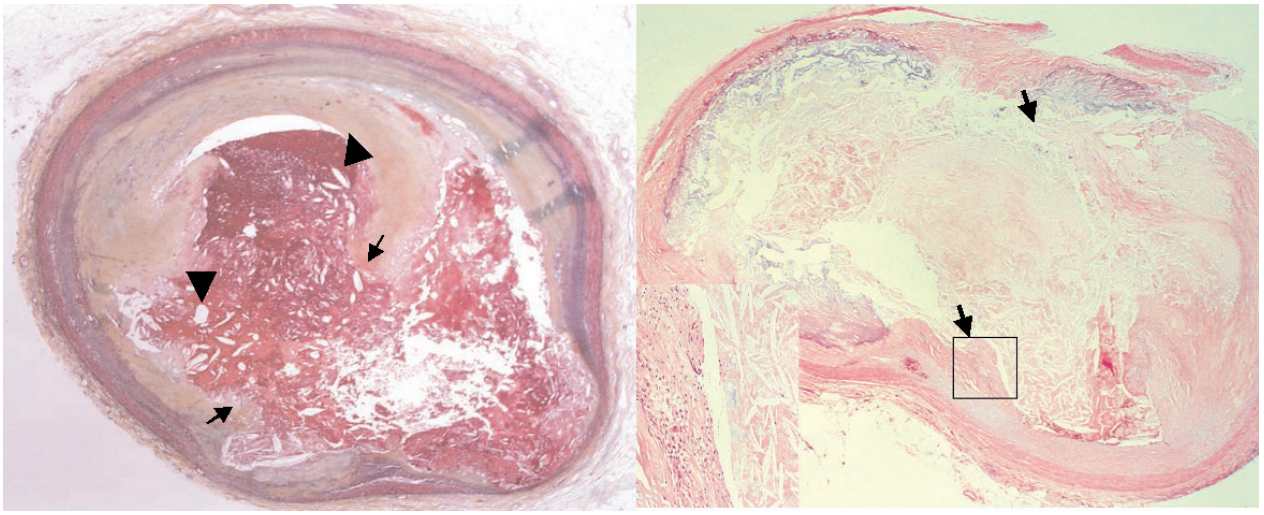


(A) (B)

FIG. 1 Examples of ruptured human plaques from patients who had died from acute myocardial infarction. (A) A light micrograph of a typical ruptured atherosclerotic plaque demonstrating dense cholesterol clefts representative of crystals (arrow heads) seen extruding through the torn cap (arrows). A fresh thrombus is noted occluding the arterial lumen (hematoxylin/eosin; magnification 147×). (B) Another example of plaque rupture from a postmortem specimen illustrating dense cholesterol crystal mass protruding into the arterial lumen and obliterating the lumen without thrombus. The plaque cap is ruptured (arrows at frayed cap edges) with crystals at the edges and filling all the lumen spaces (hematoxylin/eosin; magnification 134×). Crystals are noted to be extending into the arterial lumen (insert magnification 334×).

matous plaques and are usually present abundantly in ruptured plaques as illustrated by the examples in Figure 1, yet their role in plaque rupture is unknown. Since plaque rupture is a dynamic process, we evaluated the potential role of cholesterol crystals in acute cardiovascular events. We hypothesized that soft atheromatous plaque with large lipid deposits is supersaturated with cholesterol that can undergo physical transformation from a liquid to a solid crystal state. The growing crystals can then expand within the confined space of the plaque and damage the fibrous cap. This study was designed to demonstrate that conversion of the physical state of cholesterol from a liquid to a solid crystal state can cause extensive damage that can rupture biological membranes.

Methods

Cholesterol Crystals

Purified cholesterol powder (5-cholesten-3β-Ol; 3β-hydroxy-5-cholestene [$C_{27}H_{46}O$]: molecular weight = 386.7; 95–98% pure. Sigma, St. Louis, Miss., USA) was melted in graduated cylinders using a heating gun (HAG 1400-U, GarTec, Baden, Germany), then allowed to cool to room temperature. Volume change over time was measured by a stop watch and images of cholesterol crystal growth obtained using a digital video camera (Digital Video Camera, Model no. GR-DVL720U, JVC, Tokyo, Japan). The time from the earliest detection of opacification in the clear liquid cholesterol to full crystallization was recorded. Volume changes were calculated by subtracting the initial measured meniscus level of liquid cholesterol from the maximal peak of crystals formed. The percent volume changes over time during crystallization were then calculated. Four separate experiments were conducted using different amounts of cholesterol powder chosen arbitrarily (1, 1.5, 2, or 3 g), each repeated six times and averaged to measure the crystallization parameters of cholesterol.

Membranes

Six New Zealand White rabbits and one Sprague Dawley rat were euthanized using 1 ml of euthasol (DelMarva Labs, Midlothia, Va., USA) intravenously and the thoracic and abdominal cavities were exposed. The mesenteric membrane and pericardium were dissected gently, removed, and placed in normal saline solution. These tissues were chosen because of their close similarity in composition and thickness to the fibrous plaque cap. Animal procedures were performed according to Michigan State University's Animal Care and Use Committee-approved protocol. Cholesterol crystals were grown in 4 ml glass test tubes with a membrane spread taut over the tube orifice. Three 1-mm diameter capillary tubes were cut to a height equal to that of the test tube and then placed in the center of each tube to enhance the contact surface area for cholesterol. This also simulated other filler materials present in the plaque. Cholesterol powder (4 g) was placed in a graduated cylinder, melted using the heat gun, and then poured into the test tube, filling it to the rim. The liquefied cholesterol was then allowed to cool to room temperature. Once the crystallization started, the membrane was quickly spread taut over the tube orifice and secured by wrapping a 4-0 silk tie around the tube, creating a confined space. After crystallization, the

membrane surfaces were then inspected by direct visualization and under a dissecting microscope (Wild M7, Heerbrugg, Switzerland) and photographed by a digital camera (EOS-Digital Rebel, Cannon, Tokyo, Japan).

Light Microscopy

Biological membrane specimens were cut and embedded in paraffin on edge. Serial tissue sections (7 μm thick) were then mounted on glass slides. The sections were then stained with hematoxylin and eosin or Masson's trichrome stains. The membranes were examined and thickness measured using a light microscope and a calibrated graticule eye piece (Ernst Leitz, GmbH, Wetzlar, Germany). Phase contrast microscopy was performed using a Microphot FX microscope (Nikon Corp., Tokyo, Japan).

Electron Microscopy

The membrane samples were prepared for scanning electron microscopy (SEM) by fixing overnight in 4% glutaraldehyde (Fisher Scientific, Pittsburgh, Pa., USA) with 0.1 M phosphate buffer (pH 7.4). Samples of 5–10-mm long tissue segments were subjected to critical point drying in liquid CO_2, mounted on stubs, and goldcoated in a sputter coater. The intimal surface was then examined using a JEOL SEM (Model JSM-6300F, JEOL Ltd., Tokyo, Japan). Transmission electron microscopy was performed after tissue selection on 1-μm sections stained with toluidine blue. Thin sections were then stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Model JEM 100-CX, JEOL, Japan).

Statistical Analysis

GraphPad Prism (GraphPad Software, San Diego, Calif., USA) was used for statistical analysis of group mean data. Measured data were reported as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used for initial analysis of the data, based on the standard presumption that linear measures such as those collected here follow a normal distribution. Post-hoc testing was performed using a two-tailed Tukey's test to control error rate for multiple comparisons. Microsoft Excel (Microsoft Corp., Redmond, Wash., USA) was used to perform regression analysis between the amount of cholesterol and crystal growth achieved and the rate of crystal growth. In all cases a p value < 0.05 was used as the criterion of statistical significance.

Results

Cholesterol Crystallization and Volume Change

To evaluate our hypothesis, we needed to demonstrate that cholesterol will expand after a change in its physical state from liquid to solid. Purified cholesterol powder ($C_{27}H_{46}O$) was placed in 10-ml graduated cylinders, melted to a liquid using a heat gun, and then allowed to cool to room temperature. The crystals were then examined by light microscopy and SEM and photographed at various magnifications.

After the cholesterol powder was melted to a liquid, the meniscus level was registered and the cylinder was allowed to cool down. The liquid in the cylinders became fully opacified within 2 min and then complete crystallization growth occurred after an additional 3 min. The crystal mass consistently rose above the marked liquid meniscus demonstrating a significant volume expansion after crystallization (Fig. 2A–C). The point of maximal crystal growth constituted up to a 45% increase over the original meniscus level, resulting in rapid volume expansion (Fig. 3A–D). There was a direct linear relationship between the amount of cholesterol and both the extent and rate of crystal growth ($r = 0.98$; $p < 0.01$, $r = 0.99$; $p < 0.01$, respectively) (Fig. 3E, F). Although the percent peak crystal growth change was not significantly different among all the four weights of cholesterol tested, the level of peak growth of crystallization was significantly greater for 3 g compared with 2 g ($p < 0.01$), 1.5 g ($p < 0.001$), and 1.0 g ($p < 0.001$).

Membrane Rupture by Growing Cholesterol Crystals

To evaluate the potential damage to a biological membrane, cholesterol crystals were grown in 4 ml glass test tubes with the open end covered by smooth, thin, taut membranes.

The clear liquid cholesterol started to crystallize from the bottom of the tube and then quickly opacified into a white milky texture within 2 min of initiation. Thereafter, cholesterol crystals developed rapidly to form large clusters that occupied the space above the tube orifice. As the cholesterol crystal clusters grew to occupy the space above the edge of the tube, they pushed, distorted, and tore the membrane surface while protruding through it (Fig. 4A, B). These findings were noted in all 12 membrane-covered test tubes studied. Most stretching and tearing occurred where the membrane contacted the circumferential edges of the test tube.

Viewed by light microscopy and SEM, two predominant crystal geometries were noted: elongated sharp needle-tipped crystals (Fig. 5A–C) and flat square or rhomboid-like crystals (Fig. 5D). The flat crystals were present mostly at the base of the growth, and sharp-edged crystals were usually noted at the leading edge of the crystal growth. The elongated sharp-tipped crystals had shafts that frequently measured 5 μm in diameter at the midsection and 0.7 μm at the tip. Viewed using a birefringence microscope with polarized light, the crystals appeared to have a feather-like appearance with a plethora of colors (Fig. 5E). Using SEM, it was possible to demonstrate the tips of sharp crystals protruding through the membrane surface (Fig. 6A–F). Histology of the membrane on cross-section revealed the membrane as homogeneous and between 20 and 40 μm thick. It stained blue with trichrome, indicating a high collagen content. Also, transmission electron microscopy of the membranes demonstrated a predominance of collagen.

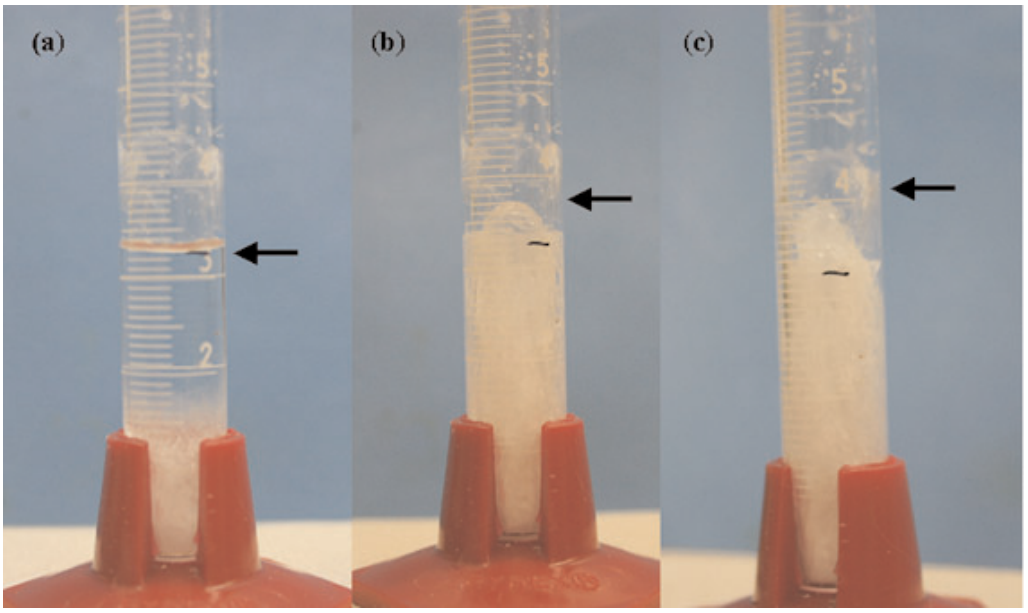


FIG. 2 Gross view of cholesterol crystal growth in a graduated cylinder demonstrating volume increase. (A) Graduated cylinder demonstrating the meniscus level of liquid cholesterol (arrow and ink mark) with early crystallization starting at the base of the graduated cylinder, (B) after intermediate level of crystal growth (arrow), and (C) at final level of crystal growth achieved (arrow).

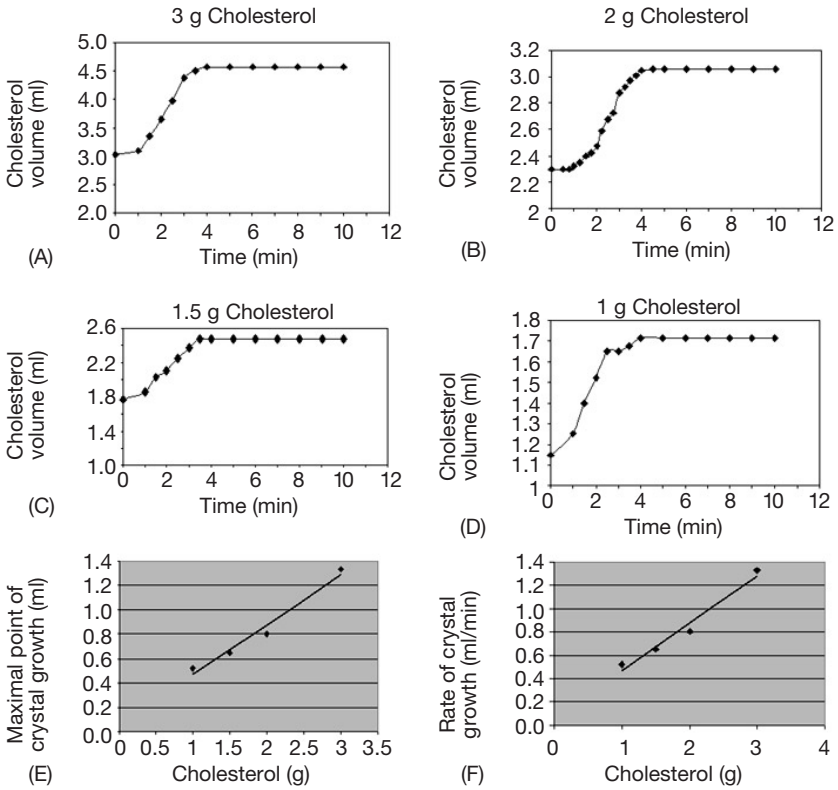
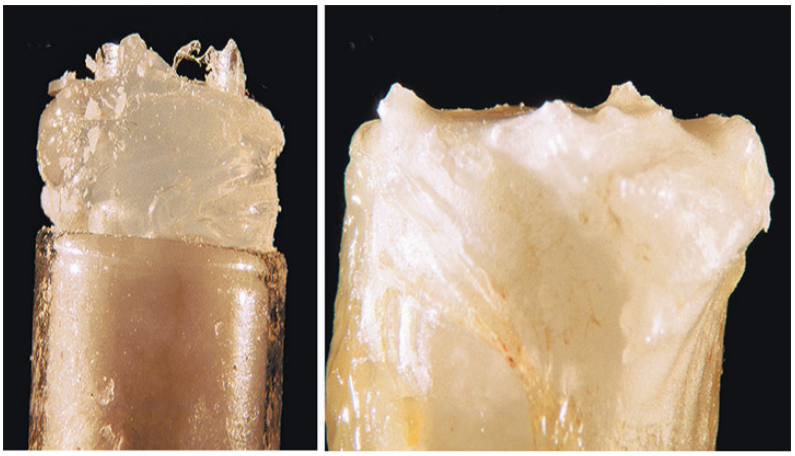
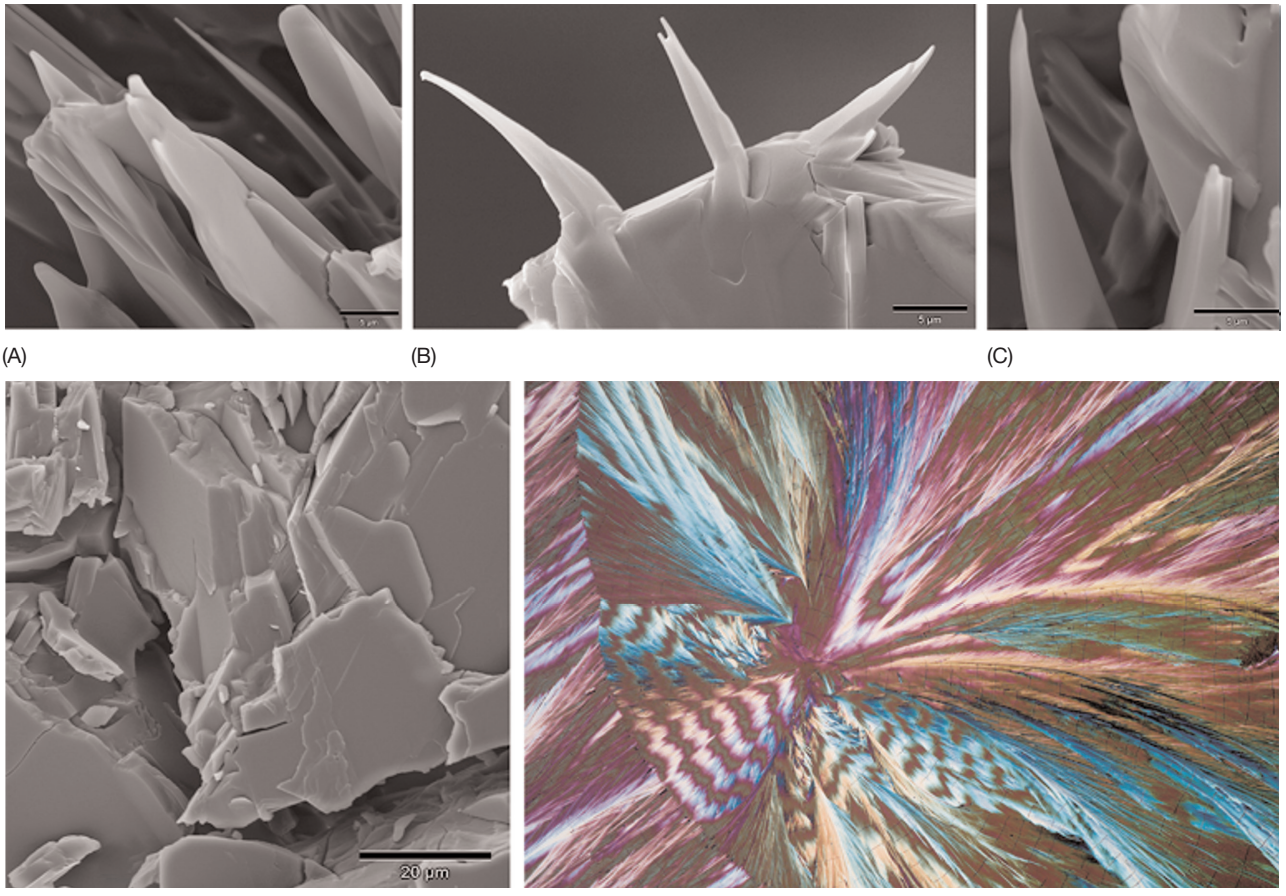


FIG. 3 Graphics of cholesterol crystal formation over time with a linear relationship between the amount of cholesterol and both extent and rate of crystal growth. These graphics demonstrate a rapid crystal growth in 3 min for (A) 3 g, (B) 2 g, (C) 1.5 g, and (D) 1 g. The maximal point of crystal growth ranged from 35–45% for all four weights. (E) This graph demonstrates a highly correlated linear relationship between cholesterol weight and maximal point of crystal growth ($r = 0.98$; $p < 0.01$). (F) This graph demonstrates a highly correlated linear relationship between cholesterol weight and rate of crystal growth ($r = 0.99$; $p < 0.01$).



(A) (B)

FIG. 4 Gross view of cholesterol growth beyond the tube edges and membrane distortion. (A) Test tube demonstrating marked increase in the cholesterol volume expanding beyond the rim of the glass tube while maintaining the shape of the tube. Large sharp crystals are seen protruding above the cholesterol column. (B) Membrane surface is irregular, demonstrating stretching and bulging of the membrane overlying the expanded cholesterol crystals.



(A) (B) (C)

(D) (E)

FIG. 5 Scanning electron microscopy image of cholesterol crystals that formed after melting the powder and cooling to room temperature. The crystals had two major types of geometry, sharp needle or jagged tipped forms (A, B, C) and flat hexagonal forms (D). Viewed by phase contrast microscopy, it could be seen that crystals form feather-like patterns that diffract light at various wavelengths generating many colors including red, white, blue, green, and yellow (E).

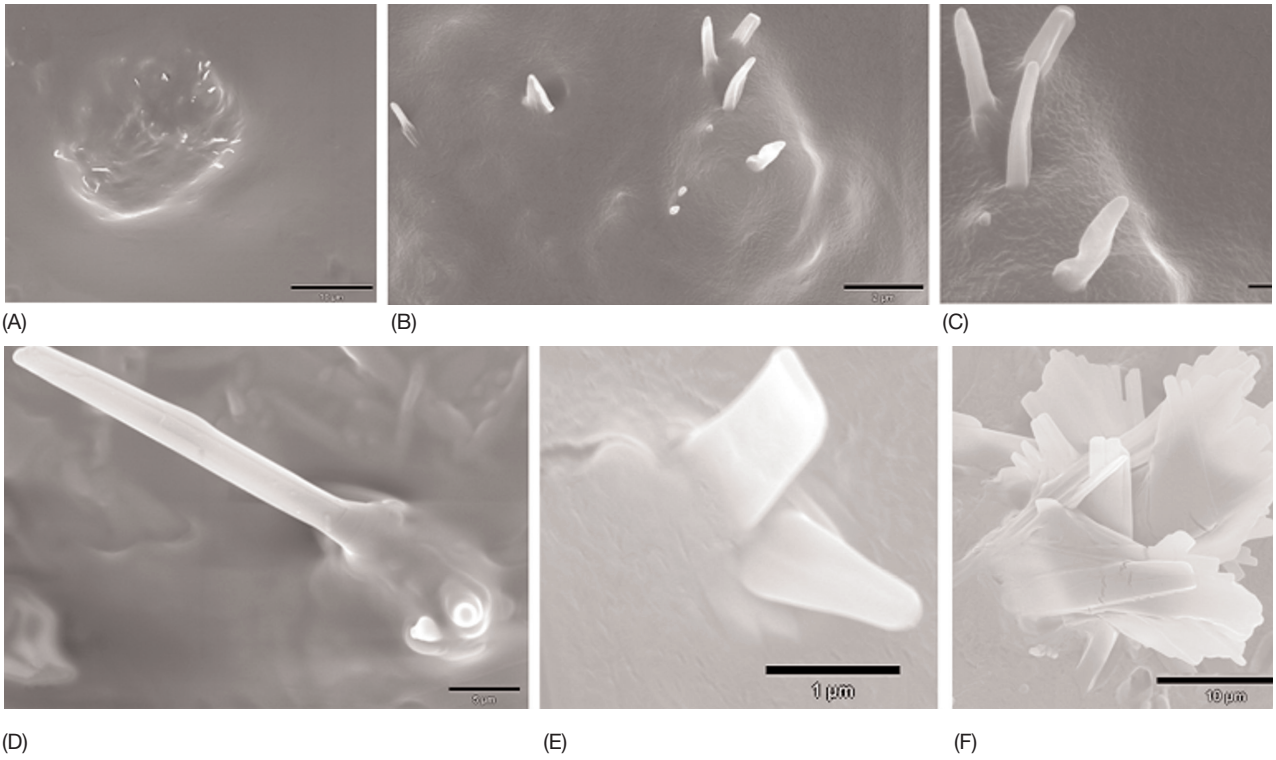


FIG. 6 Scanning electron microscopy image (SEM) of membranes demonstrating penetration of the membrane by cholesterol crystals following crystallization. (A, B, C) SEM demonstrating sharp tips of cholesterol crystals cutting through the membrane at the same site using three levels of magnification. (D, E, F) Additional examples from other sites also demonstrating various crystal shapes protruding through the membrane.

Ruptured human plaques have been reported to have a mean plaque cap thickness of 23 ± 19 µm, and plaques vulnerable to rupture have been recognized to have a plaque cap thickness of < 65 µm.[14]

Discussion

In this study, we have demonstrated that cholesterol occupies more space in a solid crystal state than as a liquid. This critical observation has many potential biological implications. Specifically, the presence of free cholesterol within a lipid-rich atheromatous plaque provides the ideal environment in which expansion and disruption in a confined space can lead to plaque rupture. Although histological images of arteries at postmortem have frequently demonstrated clusters of cholesterol crystals at sites of plaque rupture, thus far these have not been implicated as a major contributor to plaque rupture.[15–17] Our experiments demonstrated that the physical transformation of cholesterol from a liquid state into sharp needle-like crystals resulted in a maximal peak growth of up to 45%. Within the confined space of an atherosclerotic plaque and in the presence of a thin cap, this could lead to plaque cap damage including rupture and/or erosion. The exposure of plaque content to the circulating blood elements stimulates thrombus formation. In addition, the pattern of concentrated crystal distribution noted at the tube edges in this study coincides with the observations that plaques also frequently rupture at the edges.[18, 19] The membrane distortion, tears, and protrusion of cholesterol crystals through the surface noted in this study constitute evidence of damage that would be expected to cause plaque rupture and/or erosion. In this report we demonstrate two potentially injurious effects of cholesterol crystallization. One effect is the force of the crystal growth leading to gross membrane damage by distortion and tearing; this simulates plaque rupture. The second effect is the extrusion of cholesterol crystals through the membrane that can cause local endothelial injury; this simulates plaque erosion.

Alteration in the plaque's physical conditions may promote cholesterol crystallization. These could include low temperature and changes in pH, high transmural pressure, and increased local tissue concentration of cholesterol. Despite earlier investigations of physical properties of cholesterol crystals, none has related crystal formation to plaque rupture and/or erosion.[9–12] In fact, some have even described cholesterol crystals to be relatively "inert."[11] Although some investigations have implicated macrophage apoptosis by cholesterol crystals, those studies have focused primarily on intracellular cholesterol and comparison of oxidized to nonoxidized cholesterol as a cytotoxic agent.[20, 21] A large lipid pool has been considered to be a major landmark of ruptured plaque, and cholesterol deposits are predominantly seen in extracellular

pools.[5] This can constitute a critical amount of cholesterol concentration that is needed for the supersaturation required to initiate crystallization. However, as we have demonstrated, once crystallization starts it proceeds rapidly and forcibly to damage overlying membranes.

Thus far, inflammation has been considered the primary initiator of plaque disruption based on interactions between macrophages and various collagenases that can weaken the fibrous cap;[6–8] however, the exact mechanics leading to rupture of the fibrous cap have been elusive. In this study, we provide evidence that crystallization of cholesterol can rupture biological membranes by stretching and tearing. This finding should come as no surprise based on the high frequency of cholesterol crystals seen extruding at plaque rupture sites in human pathologic specimens (Fig. 1). Moreover, it would be expected that the process of cap injury will stimulate a localized inflammatory response that may explain the accumulation of macrophages and other inflammatory cells at the edges of ruptured plaques.[5] However, the role of cholesterol crystals has been greatly underestimated, probably because methods of tissue processing for histology use strong solvents that can readily dissolve and wash away the cholesterol crystals, leaving behind imprints called clefts.

In addition to the human pathologic studies, various animal models have been used to evaluate plaque rupture.[22, 23] However, these studies have also been limited by tissue processing that dissolves cholesterol crystals. Although it would be difficult to image these events because cholesterol crystals are too small for resolution by current clinical imaging techniques, detection of large lipid pools may still provide an adequate surrogate for potentially unstable lesions in the screening of patients at risk for cardiovascular events. In this study, we have demonstrated that larger amounts of cholesterol produced greater absolute volume and rate of crystal expansion, suggesting a higher potential for intimal damage. In a recent study, using an atherosclerotic rabbit model of plaque disruption and thrombosis, we demonstrated that a greater amount of cholesterol content in the arterial wall was associated with increased thrombosis.[24] Similarly, both clinical and postmortem studies have demonstrated that an increased atherosclerotic plaque load was associated with more cardiovascular events.[25, 26] All these observations reinforce the concept that a large load of cholesterol deposit in the artery contributes toward a higher frequency and severity of acute events.

Our findings are not only unique but also consistent with the unpredictable and usually random occurrence of plaque rupture and thrombosis noted clinically; that is because the timing of crystallization depends on many local physical factors. In fact, the majority of myocardial infarctions occur without an identifiable trigger.[27] Furthermore, the increased incidence of myocardial infarction in patients with high blood pressure would suggest that elevated pressure may influence the crystallization process. The use of statins has been shown not only to lower cholesterol but also to reduce cholesterol crystal formation.[28] Also, our observations are consistent with some of the pleotropic behavior of statins associated with an early reduction in acute cardiovascular events.

Study Limitations

Limitations of this study include the in vitro setting of the experiments. In addition, we used melted liquid cholesterol, while in humans there is a distribution of cholesterol in various states including liquid, liquid crystal, and solid crystal.[9] The biological membranes used in our study were not plaque caps, but we demonstrated that they were very similar to ruptured and vulnerable human plaque caps by their rich collagen composition and thickness.[14] Nevertheless, the observations in our study are compelling and suggest that the role of cholesterol as a direct mechanical injurious agent deserves further attention.

In this study, we have also developed a simple yet effective model to study the impact of cholesterol crystals on biological membranes in an in vitro setting. In the absence of similar models to evaluate plaque rupture, this proposed approach allows further studies to investigate methods for interrupting the damage of biological membranes by growing cholesterol crystals.

Conclusion

We have demonstrated that an alteration in the physical state of cholesterol from a liquid to a solid crystal state causes damage to biological membranes and hence has a strong potential to induce plaque rupture in humans. This is based on the forceful and rapid volume expansion generated by the formation of sharp-edged crystals that can damage the plaque cap. Other approaches will be needed to confirm these findings, but this study opens a new venue for the evaluation of plaque rupture and/or erosion that is based on mechanical damage induced by the physical state changes of cholesterol within atherosclerotic plaques. Also, this study provides a new approach to target plaque rupture by methods that alter physical characteristics of cholesterol crystal formation. Further understanding of the mechanical forces causing plaque rupture can lead to novel modalities of treatment to stop crystal formation and prevent plaque rupture and cardiovascular events.

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References

1. Constantinides P: Plaque fissures in human coronary thrombosis. *J Atheroscler Res* 1996;6:1–17

2. Davies MJ, Thomas AC: Plaque fissuring: The cause of acute myocardial infarction causing sudden ischaemic death, and crecendo angina. *Br Heart J* 1985;53:363–373
3. Van der Wal AC, Becker AE, van der Loos CM, Das PK: Site of intimal rupture or erosion of thrombosed coronary artherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994;89:36–44
4. Arbustini E, Dal Bello B, Morbini P, Burke AP, Bocciarelli M, Specchia G, Virmani R: Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. *Heart* 1999;82:269–272
5. Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J: Risk of thrombosis in human atherosclerotic plaques: Role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J* 1993;69:377–381
6. Lendon CL, Born GVR, Richardson PD: Atherosclerotic plaque caps are locally weakened when macrophages density is increased. *Atherosclerosis* 1991;87:80–87
7. Henney AM, Wakeley RP, Davies MJ, Foster K, Hembry R, Murphy G, Humphries S: Localization of stromelysin gene expression in atherosclerotic plaques by in situ hybridization. *Proc Natl Acad Sci U S A* 1991;88: 8154–8158
8. Galis ZS, Sukhova GK, Lark MW, Libby P: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 1994;94:2493–2503
9. Lundber B: Chemical composition and physical state of lipid deposits in atherosclerosis. *Atherosclerosis* 1985;56:93–110
10. Elisabeth N, Joseph C, Ittyachen MA: Growth and micro-topographical studies of gel grown cholesterol crystals. *Bull Mater Sc* 2001;24:431–434
11. Small DM: Progression and regression of atherosclerotic lesions. Insights from lipid physical biochemistry. *Arteriosclerosis* 1988;8:103–129
12. Kellner-Weibel G, Yancy PG, Jerome WG, Walser T, Mason RP, Phillips MC, Rothblat GH: Crystallization of free cholesterol in model macrophage foam cells. *Arterioscler Thromb Vasc Biol* 1999;19:1991–1998
13. Stillinger FH: Eccentricities of an everyday substance. *Nature* 1999;401: 850–851
14. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R: Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med* 1997;336:1312–1314
15. Schaar JA, Muller JE, Falk E, Virmani R, Fuster V, Serruys PW, Colombo A, Stefanadis C, Casscells SW, Moreno PR, Maseri A, van der Steen AFW: Terminology for high-risk and vulnerable coronary artery plaques. *Eur Heart J* 2004;25:1077–1082
16. Moreno PR, Purushothaman DR, Fuster V, Echeverri D, Truszczynska H, Sharma SK, Badimon JJ, O'Connor WN: Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: Implications for plaque vulnerability. *Circulation* 2004;110:2032–2038
17. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM: Lessons from sudden coronary death: A comprehensive morphological classification scheme for atherosclerotic lesions. *Atheroscler Thromb Vasc Biol* 2000;20: 1262–1275
18. Richardson PD, Davies MJ, Born GVR: Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet* 1989;21:941–944
19. Loree HM, Kamm RD, Stringfellow RG, Lee RT: Effects of fibrous cap thickness on peak circumferential stess in model atherosclerotic vessels. *Circ Res* 1992;71:850–858
20. Kellner-Weibel G, Jerome WG, Small DM, Warner GJ, Stoltenborg JK, Kearney MA, Corjay MH, Phillips MC, Rothbalt GH: Effect of intracellular free cholesterol accumulation on macrophage viability: A model for foam cell death. *Arterioscler Thromb Vasc Biol* 1998;18:423–431
21. Geng Y-J, Phillips JE, Mason RP, Casscells SW: Cholesterol crystallization and macrophage apoptosis: Implication for atherosclerotic plaque instability and rupture. *Biochem Pharm* 2003;66:1485–1492
22. Abela GS, Picon PD, Friedl SE, Gebara OC, Miyamoto A, Federman M, Tofler GH, Muller JE: Triggering of plaque disruption and arterial thrombosis in an atherosclerotic rabbit model. *Circulation* 1995;91:776–784
23. Rekhter MD: How to evaluate plaque vulnerability in animal models of atherosclerosis? *Cardiovascular* Res 2001;54:36–42
24. Ma H, Huang R, Hage-Korban E, Maheshwari A, Qiao X, Abela GS: Arterial wall cholesterol content directly correlates with the extent of arterial thrombosis. *FASEB J* 2003;8:LB38
25. Goldstein JA, Demetriou D, Grines CL, Pica M, Shoukfeh M, O'Neill WW: Multiple complex coronary plaques in patients with acute myocardial infarction. *N Engl J Med* 2000;343:915–922
26. Qiao JH, Fisbein MC: The severity of coronary atherosclerosis at sites of plaque rupture with occlusive thrombosis. *J Am Coll Cardiol* 1991;17: 1138–1142
27. Culic V, Etrovic D, Miric D: Meta-analysis of possible external triggers of acute myocardial infarction. *Int J Cardiol* 2005;99:1–8
28. Mason RP, Walter MF, Jacob RF: Effects of HMG-CoA reductase inhibitors on endothelial function: Role of microdomains and oxidative stress. *Circulation* 2004;109(suppl II):II-34–41