There is general agreement that atherosclerotic plaque rupture is a major clinical problem and that finding ways to prevent it would be greatly facilitated by an animal model of plaque rupture. The article by Schwartz et al1 seeks to clarify and define the terms used in descriptions of disrupted atherosclerotic plaques, and then to apply these to murine models in order to assess their similarities to and differences from human ruptured lesions.

On the face of it this seems a reasonable thing to do, but it has some serious conceptual problems. The first is the issue of whether it is useful at all to provide definitions of disrupted plaques, especially when these are based on histopathology. The second concerns the transferability of such definitions from human to mouse. I would like also to deal with issues relating to reevaluation of existing mouse models of plaque rupture.

The Usefulness of Definitions

What do we gain from framing scientific definitions of real-world phenomena? In the context of the ruptured plaque, Schwartz et al1 argue that this would provide an agreed upon and consistent framework that would make it easier for different labs to compare and contrast their data. There is obviously virtue in this as a general principle in science, but we have to consider the possibility that in the developing field of plaque rupture it may be premature. We do not know why plaques rupture, we do not know whether “plaque erosions” are simply the adjacent manifestations of ruptures that are out of the plane of the section, and we do not know whether “plaque fissures” arise through a different set of processes to plaque ruptures. In other words, this means that we do not know whether ruptures, erosions and fissures are ontogenically similar or different.

We are therefore moving beyond the boundaries of our knowledge if we set out definitions of these terms. If it should turn out that ruptures, fissures, and erosions are all different facets of the same set of underlying biochemical processes, the existence of distinct definitions will actually be unhelpful. There are plenty of examples in the biological sciences where definitions have hindered progress—currently, the emerging field of progenitor cell biology is suffering from precisely this problem, with such cells being regularly being defined and redefined as changes are made to the identifying set of surface markers—so we should resist this laying down of what are, in effect, rules unless we have a high level of confidence that they are accurate. It is clearly difficult to argue that this is the case for disrupted plaques, and it would be prudent therefore at present to frame definitions loosely or eschew them altogether.

The problems of constraint that are caused by definitions can be illustrated in a number of ways. For example, it is suggested by Schwartz et al1 that we should not describe the appearance of, inter alia, smooth muscle as making a fatty streak more or less likely to rupture. According to their article, fatty streaks are populated exclusively by macrophages, so the appearance of any smooth muscle presumably means that it is no longer a fatty streak. Does this mean that we can then discuss its stability? This sort of confusion is the direct result of restrictive histopathologic definition.

In a second and more important example, Schwartz et al1 say that “plaque rupture” implies a structural defect in the fibrous cap that separates a necrotic core from the lumen. This itself implies that the fibrous cap can be defined as the structure that divides the necrotic core from the lumen, but we know that plaques can develop defects in their surface structures even in the absence of a necrotic core (such a histopathologic feature would be proposed to be called an erosion). It means that when there is a defect in the cap over a less mature plaque that has not yet accumulated necrotic material the term “rupture” would be unavailable: this implies a distinct pathophysiology, but we do not know whether the process of disruption is actually any different to that occurring in a “rupture”. The only thing that actually matters is that the plaque in question has a common biochemistry and physiology with ruptured plaques, but to ignore these matters and simply to exclude it on the basis of a constructed definition points out very clearly why we should resist definitions that are based on histopathology rather than on an extensive understanding of the processes underlying the pathology.

Transferability of Definitions From Human to Mouse

The definitions that have been advanced by Schwartz et al1 are based mainly on observations of human plaques. There are some definitions in current use that we would all agree cannot be transferred from human to mouse: for example, in humans a thin-cap fibroatheroma is defined as having a fibrous cap less than 65 μm thick,2 but fibrous caps in mice are always considerably thinner than this. It would be unreasonable to say on this basis that all mouse plaques are...
thin-cap fibroatheromas, because we acknowledge that different benchmarks must apply and that we must adopt different perspectives for different species. One could object that this is a poor example because it contains a quantitative criterion—namely, 65 μm—whereas the definitions of terms advanced by Schwartz et al are descriptive. Is there any problem with transferring purely descriptive definitions from human to mouse?

To answer this, it is necessary first to consider what it is we are hoping to achieve with mouse models of plaque rupture. We are seeking to learn more about the basic processes underlying plaque rupture and also to use these models as test-beds for potential therapies and interventions. Thus the usefulness of mouse models will derive from congruence with human disrupted plaques in terms of biochemistry, physiology, and pharmacology. The thing to emphasize here is that this list does not include histopathology: this is because the histopathologic appearance of a mouse plaque will not matter if the underlying causes of plaque disruption in this species are completely different. Mouse ruptured plaques could look exactly like their human equivalents but could arise quite differently and thus be uselessly misleading: we must also acknowledge that they could have exactly the same set of underlying processes but look quite different, in which case they will be very useful. Based on this logic, we should be very firm and say that histopathologic criteria should not be used to judge mouse models.

**Implications for Mouse Models of Plaque Rupture**

Definitions of various types of plaque disruption, based on their histopathologic appearances in humans, may be inappropriate in the mouse and should not simply be transferred across species. Otherwise, if the mouse lyses its tiny thrombi rapidly it may never fulfil the definition of rupture given by Virmani et al, even if its plaques actually are rupturing. This means that even accurate and potentially useful mouse models would always be open to attack for not displaying histopathologic features that are required in the definition of plaque rupture. This might hamper efforts to reduce the impact of plaque rupture on human health, because such attacks could unnecessarily impede mouse model-based research into new therapies. It is noteworthy that the new definition of plaque rupture proposed by Schwartz et al no longer requires the presence of a thrombus connecting the core to the lumen, but this would mean that post mortem mechanical disruption of the plaque could be misinterpreted as plaque rupture. Neither the definition of Virmani et al or that of Schwartz et al are fully applicable to the mouse, and serve to show the problems inherent in tight histopathologic definition of poorly understood processes.

**Evaluating Mouse Models of Plaque Rupture**

It could be argued that mine is a counsel of despair: we cannot use histopathologic criteria to assess murine models of plaque rupture, and yet we do not know enough about the biochemistry of human plaque rupture to say whether a given mouse model has a good resemblance or not. Where can we find some solid ground on which to base our assessment?

It is true that if we ignore all histopathologic criteria, then any murine system could be called a model of plaque rupture. It would not even have to develop atherosclerotic plaques. This is clearly unsatisfactory, and a pragmatic workaround is called for. We need some basis on which to assess mouse models that rejects those very unlikely to be useful, but allows for further investigation of possible improved systems. One way to do this without presuming too much about the pathophysiology of plaque rupture is to say that any mouse model that displays a visible defect in the fibrous cap over an atherosclerotic lesion, with good evidence that the defect has occurred during life, should be considered suitable for further investigation. A priority for such further investigation should be the pharmacological response of the mouse model. Statins have been shown to influence apolipoprotein E (apoE) knockout mouse plaque composition and to inhibit rupture, and they do so without lowering plasma cholesterol in these animals. They are thus at present the best available way of testing the usefulness of a putative mouse model.

**The Possibility of Artifactual Findings in Mice**

Schwartz et al urge cautious interpretation of murine plaque ruptures as reported by my own laboratory among others, suggesting that these small vessels may be prone to damage during post mortem processing that could be misinterpreted as a rupture event that happened in life. This is not a new idea. We have shared this concern ever since we first reported plaque disruptions in mice and have treated it as a testable hypothesis, making a number of predictions based on the hypothesis and then comparing these with quantitative data.

The first prediction is that defects in the fibrous cap will not be accompanied by ingress of blood cells into the plaque. In other words, defects that occur as a result of manipulation of a plaque in a pressure perfusion-fixed, excised vessel cannot result in erythrocyte entry into the lesion because there are no erythrocytes present, and in any case they would not be mobile in a fixed vessel. The operating definition of acute plaque rupture that we have used is “a visible defect in the cap... accompanied by intrusion of erythrocytes into the plaque below it”. We use this definition because we are intent on avoiding erroneous counting of artificial damage as plaque rupture. Acute plaque rupture, defined this way, is first seen in the proximal brachiocephalic artery of male apoE knockout mice after 5 weeks of fat-feeding. After 8 weeks of fat-feeding, 107 of 173 animals (62%) exhibited acute plaque rupture in the brachiocephalic artery. One week later, this proportion had fallen to 30% (11 of 37 animals), suggesting that there had been a wave of plaque ruptures with a peak around the 8-week time point. Therefore this prediction, based on the hypothesis that handling artifacts may be misinterpreted as plaque disruptions occurring during life, can be shown not to hold true. Defects in the fibrous cap are in fact frequently accompanied by intrusion of erythrocytes into the plaque below them.

A second prediction is that the rate of incidence of defects in the fibrous cap will be similar at all time points once plaques have developed, because handling injuries are not related to the stage of development of the plaque. As
described above, published data show that the incidence of defects in the fibrous cap varies markedly and significantly at different time points. For example, the incidence falls from 62% to 30% within the course of a week \( (P=0.0005). \) Therefore this prediction also fails to hold true: defects in the fibrous cap occur at a rate that is different at different time points.

The third prediction based on the handling artifact hypothesis is that the rate of incidence of defects in the fibrous cap will be independent of drug treatment, because treatments administered to mice during life cannot influence the infliction of post mortem handling damage on plaques. However, the incidence of acute plaque rupture is statistically very highly significantly reduced by treatment with pravastatin: after 40 weeks of high-fat feeding, continuous treatment with pravastatin reduced the incidence of acute plaque rupture by 86% \( (P<0.0001). \) Even when treatment was delayed until 16 weeks of high-fat feeding had already elapsed, acute plaque ruptures occurred 56% less frequently \( (P<0.0001). \) It is particularly notable that the latter effect was achieved in the absence of any significant effect on plaque size \( (\sim 6\%). \) Therefore this third prediction also fails, because defects in the fibrous cap occur at a rate that can be influenced by pharmacological treatment and this can be achieved independently of any effect on plaque size.

Before dismissing the handling artifact hypothesis, we should consider a related hypothesis also advanced by Schwartz et al. They suggest that pravastatin treatment could modify the composition of plaques in such a way as to make them less fragile and thus less vulnerable to handling damage. One could further argue that plaque growth and maturation also result in reduced fragility, accounting for the change in the incidence of plaque disruption over time in untreated animals. However, this still fails to explain the association between visible cap defects and the presence of accumulations of erythrocytes. We therefore suggest that there are sufficient published peer-reviewed data to make it safe to discard the idea that acute plaque ruptures in mice are really post mortem handling artifacts.

**Healed Plaque Ruptures and Buried Fibrous Caps**

In humans, nearly 90% of plaque ruptures are seen as previous, now healed, defects in layers in the body of the plaque. They are more common at the sites of acute plaque rupture. Elevated plasma cholesterol is significantly associated with the presence of healed ruptures by multivariate analysis, and the healed rupture sites contain a preponderance of smooth muscle cells, evidence of hemorrhage or thrombus, and layering of collagen. This collagen shows differential optical polarization when stained with picrosirius red: older collagen appears orange-yellow and newer collagen appears green. The newer collagen is seen at the site of the defect in the healed rupture.

We have described an unusual structural feature of proximal brachiocephalic artery plaques in fat-fed apoE knockout mice. These very hypercholesterolemic animals show layers in their plaques that are rich in smooth muscle cells and elastin, are statistically significantly more likely
fibrous layers is significantly related to the occurrence of acute plaque rupture but not to plaque size, and the prediction is not borne out by the available data.

The second prediction is that the presence of fibrin in the plaque should be independent of the presence of a buried fibrous cap, because fibrin would be a sign of thrombosis whereas buried fibrous caps are hypothesized to be part of normal plaque development. We have shown that immunoreactive fibrin is present at the sites of buried fibrous caps in apoE knockout mouse brachiocephalic arteries, using a goat polyclonal antibody that is not reactive with fibrinogen. This antibody (a kind gift from Dr Douglas Thompson, Department of Pathology, University of Aberdeen, UK) was unreactive with mouse fibrinogen at any dilution, but incubation of the fibrinogen with thrombin to generate fibrin resulted in immunodetection at dilutions as low as 1 in 500. The antibody also bound to clotted mouse blood, but was unresponsive to thrombin alone. Furthermore, in a series of 50 male apoE knockout mice that had been fat-fed for 8 weeks, the presence of fibrin (as assessed by bright red staining with Masson trichrome) was significantly associated with the presence of a buried fibrous cap. Of the 50 mice, 23 had buried fibrous caps at this site, and 19 of these 23 plaques were positive for fibrin. Among the 27 mice with no buried fibrous caps, 4 stained positively for fibrin (P=0.000001). We conclude that fibrin accumulation at sites of buried fibrous caps is verified by specific antibody staining, and by tinctorial criteria is statistically very highly significantly associated with the presence of buried fibrous caps. This means that the second prediction is also contradicted by the data.

The third prediction that flows from the hypothesis that buried fibrous caps are part of normal plaque development in mice is that their incidence should covary with plaque size. Studies in apoE knockout animals treated with pravastatin, or with an additional null mutation to the cathepsin S gene, show that plaque size and the number of buried fibrous caps can be modulated independently. When pravastatin treatment commenced after advanced plaques had already developed, the formation of buried fibrous caps was reduced by 36% (P<0.0001) but there was no significant effect on plaque size (−6%). In the case of cathepsin S, the incidence of buried cap formation normalized to plaque size was reduced by 37% (P=0.044) in the double knockouts. Thus the incidence of buried fibrous caps can be modulated independently of any effect on plaque size, and the data are once again at variance with the prediction.

We suggest that, on the basis of published peer-reviewed data, it seems safe to reject the hypothesis of a non–rupture-related origin for buried fibrous caps in apoE knockout mouse brachiocephalic arteries.

Summary

Definitions are a way of crystallising information, and can be very useful in terms of providing an agreed upon framework within which to compare and evaluate data from different laboratories or in different systems. However, this framework can also function as a rather inflexible constraint: observations that fail to satisfy definitions may be discounted or discarded when in fact it is the definition that is inaccurate. It is important therefore to have very strong theoretical bases for definitions, and not simply to construct them in an ad hoc fashion to fit a limited set of data. This is the serious conceptual problem with the approach recommended by Schwartz et al. They define various histopathologic manifestations of plaque disruption in such a way as to imply that they are pathophysiologically distinct, but we do not know whether this is actually the case.

This possibly rather abstract conceptual problem has serious adverse real-world consequences. By defining plaque rupture, for example, in histopathologic terms, we have a situation where the processes that cause plaque rupture and the processes that are set in train by plaque rupture—in particular, thrombosis—are combined. The consequence is that if mouse plaques—which can only accumulate tiny amounts of thrombotic material because they are so small—can lyse their thrombi rapidly, they may automatically fail to satisfy this definition of rupture despite the fact that they may actually have ruptured. This could even happen in the case of mouse model that undergoes processes completely consonant with those underlying human plaque rupture, so we run the risk by employing these definitions of condemning ourselves to never accepting any mouse model of plaque rupture. It is difficult to see how this can be of benefit to science or medicine, and it should therefore be vigorously resisted. A more flexible approach would be to ask workers to specify in papers and grants the criteria by which they classify plaque disruptions in mice, but not to insist that all laboratories use the same criteria. Further to this, for consideration as a model of plaque rupture it is only necessary that a visible defect is seen in the fibrous cap, with evidence that this has occurred in life.

Schwartz et al indicate two potential weaknesses in reports of murine disrupted plaques: that the acute plaque ruptures could really be post mortem handling artifacts, and that buried fibrous caps are not healed plaque ruptures but really an aspect of normal plaque growth. Both of these suggestions are contradicted by published data. The predictions that logically flow from them have been carefully tested, found wanting, and rejected with extraordinarily high levels of statistical confidence. We therefore suggest that these caveats are unwarranted, that the proximal brachiocephalic artery of the fat-fed apoE knockout mouse has all the hallmarks of an excellent model system for investigating the pathophysiology of plaque rupture, and that it appears to be an excellent test-bed for assessing novel interventions designed to ameliorate this serious clinical problem.

Disclosures

None.

References


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