Observation

Aspirin use is associated with higher serum concentrations of the anti-oxidant enzyme, paraoxonase-1

To the editor: Paraoxonase-1 (PON1), a high density lipoprotein (HDL)-associated enzyme, is a major determinant of the anti-oxidant, and thus the anti-inflammatory function of the lipoprotein [1]. Data from animal models and human studies are consistent with the enzyme being a risk factor for vascular disease, notably in diabetic patients [1]. Independent studies have shown that diabetic patients are deficient in serum activity of PON1 [2, 3], whilst other reports have shown an association between PON1 and insulin resistance [4, 5] that could impact on susceptibility to vascular disease [5]. Although the primary effect of PON1 is to prevent lipoprotein oxidation, its enzyme activity is sensitive to oxidative stress. Lipid oxidation products can inhibit the enzyme, whilst factors which promote oxidative stress are associated with reduced serum PON1 activity and concentration. The latter could be a factor in the lower serum PON1 levels in diabetic patients.

Aspirin has become an important member of the therapeutic arsenal for combating vascular disease [6]. Whilst its anti-thrombotic function figures prominently as a beneficial factor, other positive influences on vascular disease have been shown. These include anti-inflammatory effects, improved endothelial function, modulation of smooth muscle proliferation and anti-oxidant effects. In the latter case, the anti-oxidant capacity of aspirin has been shown to encompass serum lipoprotein oxidation [6]. Inhibition of formation of reactive oxygen species

(ROS) and scavenging of ROS are mechanisms that have been advanced to explain the effect of aspirin [6].

As outlined in a recent editorial [7], we are now beginning to identify factors that can influence serum levels activities of the enzyme. Anti-oxidants seem to have a beneficial effect, probably due to the deleterious influence of oxidation products on enzyme activity. Given the evidence for an anti-oxidant function of aspirin and its widespread use, we considered that it could influence serum activity of PON1. To test this hypothesis, we examined serum PON1 concentration and activity in a large cohort of coronary patients and control subjects as a function of aspirin use.

The study was undertaken in a cohort of coronary artery disease (CAD) patients and control subjects undergoing arteriography at the University Hospital, Geneva. All participants gave written, informed consent and the study was carried out according to the requirements of the ethics committee of the hospital. The cohort was described previously in detail [8]. Current medication, including aspirin use, was ascertained during completion of a questionnaire with an interviewer, and confirmed by reference to hospital records. A *p* value less than 0.05 was considered significant.

The characteristics of aspirin users and non-users are shown in Table 1. As could be expected, a larger proportion of aspirin users had CAD compared to non-users. Of the other cardiovascular risk factors, smoking was more prevalent in aspirin users and triglycerides were higher. The groups did not differ for the percentage of diabetic patients, serum cholesterol or HDL-cholesterol. PON1 genotype distributions were also analysed; there were no differences between the two groups for the coding region polymorphisms Q192R (*p*=0.74) and L55M (*p*=0.24) or the promoter polymorphism C(-107)T (*p*=0.44). Table 1 also gives mean or median values for serum PON1. There were differences with respect to PON1 mass and enzyme activities. In all cases, values were higher in subjects who took aspirin. When Type 2 diabetic patients alone were analysed, differences between aspirin users and non-users were of the same order of magnitude, but did not reach significance due to the small number of patients involved [non-users (*n*=55) v users (*n*=85): PON1 mass, 87.7±23.6 v 94.1±22.5 µg/ml, *p*=0.10; arylesterase activity, 73.4±28.8 v 79.0±22.0 U/ml, *p*=0.19; paraoxonase, 143.4 (71.7±507.8) v 170.5 (96.4±480.5) U/ml, *p*=0.14]. Forward, stepwise multiple regression analyses

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Table 1. Clinical and demographic characteristics of aspirin users and non-users

Parameter	Aspirin –	Aspirin +	ANOVA
Sex (M/F)	258/109	360/111	0.04
Age (y)	60.0±9.7	60.1±8.9	0.85
BMI	26.9±4.5	27.2±4.1	0.35
CAD (%)	33.3	62.4	<0.0001
Type 2 diabetes (%)	14.4	17.8	0.16
Never/Ex/Smoker (%)	29.8/43.5/ 26.6	22.4/56.1/21.4	<0.001
HT systolic	130.7±19.0	132.3±17.2	0.21
HT diastolic	78.2±10.9	79.8±12.0	0.06
Cholesterol (mmol/L)	5.77±1.25	5.71±1.15	0.42
Triglycerides (mmol/L)	1.44±0.78	1.63±1.08	0.004
HDL-cholesterol (mmol/L)	1.18±0.34	1.15±0.32	0.13
Activity ARE (U/ml)	78.37±25.0	83.4±24.58	0.003
Activity PON (U/ml)*	180.5 (84.8±475.9)	205.1 (94.6±510.7)	0.02
Concentration PON1 (µg/ml)	92.7±22.0	97.0±22.3	0.006

CAD, coronary artery disease; ARE, arylesterase activity; PON, paraoxonase activity; Never, never smoked; Ex, ex-smoker; Smoker, current smoker. ARE, arylesterase activity;

PON, paraoxonase activity. * Natural logarithm transformed prior to analysis. Values are given as means ± SD, except *, median (10±90th percentile)

were carried out to examine the independence from other parameters of PON1-aspirin associations. In models which included PON1 promoter and coding region genotypes, HDL, total cholesterol, triglycerides, age, sex, smoking, cardiovascular disease, diabetes, statin use and treatment for hypertension, aspirin emerged as an independent determinant of serum PON1 concentration (model $r^2=0.45$, aspirin $p=0.039$) and activity (arylesterase activity, model $r^2=0.38$, aspirin $p=0.008$; paraoxonase, model $r^2=0.82$, aspirin $p=0.0007$).

This study shows an association of aspirin not only with higher serum activities but also concentrations of the anti-oxidant enzyme, PON1. The association was independent of a host of other factors, which seem to impact on the enzyme. Absolute differences in serum PON1 parameters between aspirin users and non-users were similar in diabetic patients to those of the whole cohort. Given the growing evidence that decreased serum PON1 is implicated in susceptibility to vascular disease, modulated serum activities of the enzyme could be a contributory factor to the beneficial impact of aspirin on vascular disease. The data provide no information on potential mechanisms by which aspirin could influence PON1. It is nevertheless tempting to invoke oxidative stress, for which sound evidence exists linking it both to aspirin and PON1. An alternative possibility is the anti-inflammatory effect of aspirin, as serum PON1 levels concentrations are reduced during the inflammatory response in animal models.

The observational nature of this study precludes conclusions of a causal effect of aspirin on serum PON1. However, there exist cogent arguments to suggest that such a relationship is possible. Prospective studies would seem merited to assess if aspirin can increase serum PON1. In this context, aspirin can provide an inexpensive means of modulating the enzyme. The potential benefits could be of particular relevance to diabetic patients. They seem to be deficient in serum PON1 compared to non-diabetic patients, whilst the diabetic condition is suggested to increase the risk of oxidative stress.

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