

Aspirin action in Innsbruck

A True All-rounder

When it comes to vascular disease, Aspirin has traditionally been recommended for its protective effect against platelet aggregation and inflammation. Now Austrian scientists are showing that this popular drug also influences cholesterol efflux from atherosclerotic plaques.



What a night! And what a fight against thirst and the worst headache ever! What on earth did people do without the painkiller and all-round wonder drug Aspirin after jolly evenings more than hundred years ago? Experienced drinkers can only imagine desperate situations and suicidal thoughts.

However, whenever over-indulgers take Aspirin they inadvertently benefit from its other effects. Aspirin, or acetyl-salicylic acid, relieves not only self-inflicted pain and pain caused by disease, it also protects against heart attack and stroke. The exact mechanism by which Aspirin manages to reduce these atherosclerotic risks was always thought to be its ability to reduce platelet aggregation and inflammation, until Ivan Trancevski, Andreas Wehinger and other members of Andreas Ritsch's research group in Innsbruck, Austria, elegantly demonstrated a third effect. They discovered that Aspirin also influences cholesterol efflux from macrophages, reducing atherosclerotic risks (*FASEB J.*, Vol. 20, 1328-1334).

A new case for "Agent A."

Heart disease is often caused by atherosclerotic plaques. Such plaques carry macrophages, or foam cells, which express SR-BI (scavenger receptor class B type I). This receptor reduces atherosclerosis by promoting cholesterol efflux from the plaques. Harmful cholesterol is subsequently transported back to the liver by a HDL-receptor. So, business as usual for macrophages in the vascular system.

Other research groups recently indicated that Aspirin affects the expression of PPAR- α (peroxisome proliferator-activated receptor- α), which acts as a nuclear receptor in lipid metabolism. Aspirin is able to activate the transcription factor PPAR- α , which up-regulates SR-BI expression in macrophages of atherosclerotic areas.

Andreas Ritsch's group at the Department of Internal Medicine, Innsbruck, has revealed that Aspirin acts as a direct SR-BI enhancer, thus more or less assuming the daily job of PPAR- α . The Austrian scientists confirmed this mechanism by using experiments *in vitro* and *in vivo* checking SR-BI expression in cell cultures of human primary macrophages as well as in PPAR- α knockout and wild-type mice. Since PPAR- α is known to depend on COX-2 regulation, Trancevski, Ritsch *et al.* showed that, overall, Aspirin can act in a COX-independent way to mediate cholesterol efflux.

The setting

By using cell cultures of human macrophages and performing Western blots the Austrians concluded that low doses of Aspirin (up to 0.5 mM) enhanced SR-BI expression whereas high concentrations (1 mM or higher) showed the opposite effect. This result corresponds with the ability of the macrophages to uptake [3H]-labelled

By using blot experiments they observed that low levels of Aspirin act in combination with the PPAR- α activator fenofibrate, leading to higher quantities of SR-BI protein than incubation with either Aspirin or fenofibrate alone. Although treatment with both compounds had no effect on PPAR- α expression it increased its DNA binding capacity. Thus the scientists asked themselves whether better DNA binding of the transcription factor PPAR- α would lead to better SR-BI transcription. RT-PCR measurements of SR-BI mRNA in human cells gave the answer: "Enhanced PPAR- α DNA binding activity did not affect SR-BI transcription which suggests a post-transcriptional regulation of SR-BI."

Unsatisfying results usually lead to further experiments, and this is no different in Austria. So *in vivo*-tests in wild-type (WT) and knockout (KO) mice followed. Ritsch explains: "As seen in our experiments performed in WT mice, SR-BI expression in macrophages of PPAR- α KO mice was induced by *in vivo* treatment with low-dose Aspirin and decreased after treatment with high-dose Aspirin. These results indicated that PPAR- α is not required for either basal expression or induction of macrophage SR-BI by Aspirin."

Is COX involved or not?

Another question to clarify was whether the effects of Aspirin on SR-BI are mediated by inhibition of cyclooxygenase (COX). Ritsch's working group supplied the cells with sodium salicylate, the active metabolite of COX, and subsequently incubated them with two well

known COX1/2 inhibitors, ibuprofen and naproxen. The striking outcome was that neither compound had an impact on SR-BI expression. Further analysis of COX-independent effects showed no alterations that confirmed the COX-independent influence of Aspirin on SR-BI expression.



Ivan Tancevski (2nd from left), Andreas Ritsch (3rd from left), Andreas Wehinger (4th from left) and team experiencing collective "bombardment".

HDL cholesteryl esters as measured by liquid scintillation counting. Highest uptake was achieved in cells treated with 0.5 mM Aspirin whereas higher dosages yielded decreased HDL binding.

Trancevski and his colleagues were particularly interested in the role of PPAR- α .

Trancevski summarised the results as follows: "These opposite effects in dependence of dosages are pointing to two different scenarios of regulation. On the one hand, stimulatory effects of 0.5 mmol/l Aspirin as well as of fenofibrate [the PPAR- α activator] were not accompanied by changes in SR-BI mRNA levels, respectively. On the other hand, 5 mmol/l Aspirin treatment of primary human macrophages resulted in markedly decreased levels of both SR-BI protein and mRNA. This was accompanied by a decrease in PPAR- α protein concentration, suggesting a yet unknown effect of highly concentrated Aspirin on the expression of proteins involved in lipoprotein metabolism."

Chasing the unknown...

"Yet unknown effect": a good reason to hang in there. SR-BI has been shown to be post-transcriptionally regulated in murine liver cells and Trancevski's results suggest

a similar scenario in human macrophages undergoing low dose Aspirin treatment. Unfortunately, the regulating factor responsible for post-transcriptional up-regulation of SR-BI could not be defined. Plausibly, Aspirin could influence SR-BI protein stability in a PPAR- α -independent manner via an unknown protein. One possible candidate could be a target of NF- κ B, since its activity was increased upon low dose Aspirin treatment. Moreover, it is involved in numerous signalling cascades in atherosclerosis and in the initiation of foam cell formation. But this is pure speculation.

...by thorough speculation

Meanwhile, Andreas Ritsch and his group could try some tests *in vivo* in a clinical study. It seems very probable that human patients using therapeutic Aspirin express more SR-BI in atherosclerotic plaques as well. But this is an entirely different story...

DANIELA KAULFUS

One fine day in the lab...

by Leonid Schneider

**Congratulations! Nothing wrong with your pet.
It's as healthy as a mouse can be!**



**WHAT?!! Nothing wrong ?!!
Healthy ?!! I spent five years making
this damn knockout mouse!!!**

