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Author/s:

Eisen, DP;McEwen, P;Hansen, R;Pandey, S;Wallis, SC;Roberts, JA

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Low levels of salicylic acid and salicyluric acid are present in synovial fluid of patients taking aspirin at the time of knee arthroplasty surgery.

Damon P Eisen^{1,2}

Peter McEwen³

Regina Hansen³

Saurabh Pandey⁴

Steven C. Wallis⁴

Jason A. Roberts^{4,5,6,7}

Affiliations

1. The Townsville Hospital and Health Service, Angus Smith Drive, Douglas, Queensland, Australia.
2. College of Medicine and Dentistry, James Cook University, Discovery Drive, Douglas, Queensland, Australia.
3. Orthopaedics Research Institute of Queensland, Townsville, Queensland, Australia.
4. University of Queensland Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia.
5. Centre for Translational Anti-Infective Pharmacodynamics, School of Pharmacy, The University of Queensland, Woolloongabba, Queensland, Australia.
6. Department of Intensive Care Medicine and Pharmacy Department, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.
7. Division of Anaesthesiology Critical Care Emergency and Pain Medicine, Nîmes University Hospital, University of Montpellier, Nîmes, France.

Corresponding author:

Damon Eisen, College of Medicine and Dentistry, James Cook University, Discovery Drive, Douglas, Queensland, Australia.

Email: damon.eisen@jcu.edu.au

Telephone: +61 7 4781 5008

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DAMON P EISEN (Orcid ID : 0000-0002-8987-4358)

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Low doses of acetylsalicylic acid (aspirin) are recommended in some venous thromboembolism prophylaxis guidelines for total knee replacement arthroplasty surgery. As salicylic acid has antimicrobial activity it would be advantageous if aspirin's use reduced the risk of prosthetic joint infection. We measured salicylate levels in the synovial fluid of patients taking long term low dose aspirin who were having knee arthroplasty surgery. Low levels of salicylic and salicyluric acids were present in synovial fluid immediately pre surgery. Acetylsalicylic acid was not measurable in synovial fluid. The measured salicylic acid levels were lower than those previously shown to have antibacterial activity against any of the bacteria that typically cause prosthetic joint infection.

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Acetylsalicylic acid (aspirin) is widely used for cardioprotection and stroke prevention. Some venous thromboembolism (VTE) prophylaxis guidelines for total knee replacement arthroplasty surgery now include low-dose aspirin. (1)

Salicylic acid, the main aspirin biometabolite, may reduce the risk of post-operative arthroplasty wound infections including those that may involve the prosthesis (2) as it is reported to have in vitro antimicrobial and antibiofilm activity. (3) As such, it is important to understand the pharmacokinetics of salicylic acid and other aspirin metabolites, in the synovial fluid to assess for potential anti-infective effects.

Patients undergoing elective total knee arthroplasty surgery that were long term users of aspirin were approached for informed consent for participation in this study. While aspirin is not part of Australian VTE prophylaxis guidelines, the study surgeon's practice is to continue it perioperatively. Samples of blood were taken into EDTA tubes at induction of anaesthesia. Shortly thereafter, prior to surgical incision into the knee joint to avoid gross contamination with blood, synovial fluid samples were aspirated percutaneously. Samples were stored on ice in the operating theatre. Plasma and synovial fluid were then stored at -80°C .

Total concentrations of acetylsalicylic acid, salicylic acid, gentisic acid and salicyluric acid in plasma and synovial fluid were measured by a UHPLC-MS/MS (ultra-high performance liquid chromatography-tandem mass spectrometry) method on a Nexera2 UHPLC system coupled to a 8040 triple quadrupole mass spectrometer (Shimadzu Kyoto, Japan).

Samples were spiked with stable isotope labelled salicylic and salicyluric acid internal standards, and salicylates were extracted from the biological samples with ethyl acetate:diethyl ether (4:1).

The stationary phase was a Kinetex C8 100 x 2.1 mm (1.7 μm) analytical column (Phenomenex, Torrance, USA) and the mobile phase was a gradient of acetonitrile and water containing 0.1% formic acid. Detection was multiple reaction monitoring of negative mode electrospray at m/z of 178.95 \rightarrow 137.00 (acetylsalicylic acid), 137.05 \rightarrow 93.00 (salicylic acid), 153.15 \rightarrow 108.00 (gentisic acid), 194.20 \rightarrow 93.05 (salicyluric acid) 141.10 \rightarrow 97.05 ($^2\text{H}_4$ -salicylic acid) and 197.00 \rightarrow 152.10 ($^{13}\text{C}_2, ^{15}\text{N}$ - salicyluric acid).

Calibration range was 0.02 to 2 $\mu\text{g}/\text{mL}$ for acetylsalicylic acid, 0.01 to 20 $\mu\text{g}/\text{mL}$ for salicylic acid and salicyluric acid, and 0.1 to 20 $\mu\text{g}/\text{mL}$ for gentisic acid. Precision and accuracy were measured at three concentrations for each analyte, and were within 15.8% for salicylic acid,

within 13.1% for gentisic acid, and within 13.3% for salicylic acid. Acetylsalicylic acid degrades rapidly and precision and accuracy were within 30.8%.

Descriptive statistics were determined using GraphPad Prism (GraphPad Software). Ethics approval was granted by the Mater Hospital Health Services North Queensland Human Research Ethics Committee (MHS20170808-01).

Twenty-eight subjects were recruited into the study between February and December 2018. Eleven of the enrolled subjects stopped their aspirin preoperatively despite their surgeon's recommendation that they continue it. Two of the enrolled participants did not proceed to surgery because of logistical issues or emergent medical conditions. Seventeen participants were taking aspirin at the time of arthroplasty surgery as planned. In the case of 14 participants, paired samples of blood and synovial fluid were available for analysis. In the other three participants, one or other sample was not collected.

All the participants were taking a daily aspirin dose of 100mg. The median age was 68 years and 14 were male. Salicylic and salicylic acids were detected in synovial fluid and plasma at low concentrations as shown in Table 1 and Figure 1. The ratio of synovial fluid to plasma concentrations are also shown in Table 1 and Figure 1. No acetylsalicylic or gentisic acid were detected in any of the samples.

Aspirin is frequently used as for secondary prophylaxis of stroke and cardiac events in the population who are having joint arthroplasty surgery. While aspirin use had previously been routinely suspended by orthopaedic surgeons prior to arthroplasty this practice has now changed. Aspirin has been shown to have similar efficacy to low molecular weight heparin (4) or novel oral anticoagulant (5) usage and the same or reduced risk of post-operative haemorrhage. (4)

Aspirin is rapidly metabolised to salicylic acid via hydrolysis with further modification resulting in the formation of salicylic and gentisic acids which are excreted in the urine. (6) This biotransformation pathway explains why no acetylsalicylic acid was found in the plasma or synovial fluids. Gentisic acid is a minor metabolite of aspirin and, with the low concentrations of salicylic acid present in patients taking low-dose aspirin, it is not unexpected that it was not detectable.

Salicylic acid has been shown, *in vitro*, to have some antimicrobial activity. (3) This may be of relevance if infection of arthroplasty devices could be inhibited by aspirin usage in the perioperative period. The concentration of salicylic acid required to inhibit *Staphylococcus*

aureus (150 µg/ml) (7) and *Klebsiella pneumoniae* (50 µg/ml) (8) are within the pharmacological range of attained when aspirin is ingested in anti-inflammatory doses (150 – 300 µg/ml). (9) Additionally, salicylic acid has demonstrated in vitro activity against biofilms formed by *Staphylococcus epidermidis* (140 µg/ml), (10) *Escherichia coli* (10 µg/ml)(11) and *Pseudomonas aeruginosa* (10 µg/ml). (12)

However, in this pragmatic study we have shown that the steady state concentration of salicylic acid found in synovial fluid of a convenience sample of patients prescribed aspirin long-term prior to elective total knee replacement surgery, was two to three orders of magnitude lower than these minimum inhibitory concentrations.

The high synovial fluid concentrations of salicylic acid (86 µg/ml) measured in patients taking anti-inflammatory doses of aspirin (4.2 g/day) (13) would have potentially inhibited biofilm production by bacteria such as *S. epidermidis*, a common cause of prosthetic joint infection. To date there are no published data on the salicylic acid concentration required to inhibit *S. aureus* biofilms. Using the *in vitro* biofilm inhibiting concentrations for *S. epidermidis* and Gram negative bacteria as a guided we conclude that the salicylic acid concentrations that we have measured in synovial fluid would not have activity against *S. aureus* biofilms.

Were salicylic acid to have been found to be in higher concentrations in synovial fluid, patients continuing on aspirin after surgery may potentially have benefited from reduced risks of post-operative wound infection. The antimicrobial effects of salicylic acid would not be expected to enhance perioperative antibiotics but could be useful in prevention of infection due to resistant organisms. Prolonged antimicrobial effects after clearance of perioperative antibiotics could have been beneficial.

Aside from direct antimicrobial effects, aspirin may act on biofilms by interfering in the interaction between *S. aureus* and platelets. In the case of infective endocarditis, an intensively studied biofilm mediated disease, platelets immobilised on the surface of damaged heart valves then mediate adherence of *S. aureus*, a crucial step in the development of bacterial vegetations. (14)

Low concentrations of salicylic acid and salicyluric acid are measurable in the synovial fluid of patients having elective total knee replacement surgery who are taking aspirin preoperatively. While the ratio of synovial fluid to plasma concentrations of salicylic acid are at least 50%, the concentrations measured would not be predicted to have any antimicrobial,

particularly antibiofilm effect. If aspirin is to have any effect in reducing prosthetic joint infection it would be indirect and through a lower risk of post-operative haemorrhage and reduced interaction between platelets and bacterial pathogens including *S. aureus*.

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All authors report no conflict of interest.

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Table 1. Salicylic and salicyluric acid levels in synovial fluid and plasma. The ratio of synovial to plasma salicylate levels is also shown.

	Synovial fluid		Plasma		Ratio synovial fluid : plasma	
	SA†	SU‡	SA	SU	SA	SU
Min - max	0 - 3.3	0 - 0.83	0.017 - 10	0 - 2.6	0 - 2.7	0 - 0.99
Median (IQR [§])	0.59 (0.13 - 1.3)	0.073 (0 - 0.24)	0.67 (0.19 - 2.4)	0.22 (0.011 - 0.49)	0.54 (0.2 - 0.93)	0.22 (0 - 0.99)

† Salicylic acid

‡ Salicyluric acid

§ Interquartile range

Figure legend

Figure 1. Salicylate levels in; synovial fluid A and plasma B. Ratio of synovial to plasma salicylate levels C.

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Figure 1.

