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DEVELOPMENT OF A HIGHLY SENSITIVE AND SPECIFIC

POLYCLONAL ANTIBODY TO SALICYLURIC ACID: POTENTIAL APPLICATIONS IN MONITORING OF ASPIRIN USE OR MISUSE?

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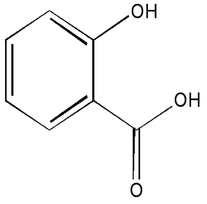
Introduction

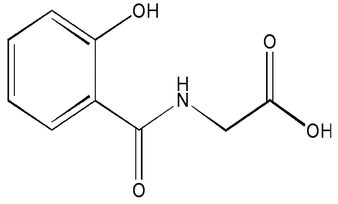
Aspirin is one of the most used drugs, indicated for the treatment of fever and pain and also used clinically to treat stroke, myocardial infarction, angina, colorectal cancer and rheumatoid arthritis. Its effectiveness as a therapeutic drug is constrained by a narrow concentration range. Once ingested, aspirin is rapidly hydrolysed to salicylic acid whose main metabolite is salicyluric acid, which makes up approximately 75% of the excreted product.[1] As the level of salicylic acid increases, the urinary excretion concentration of salicylic acid increases relative to salicyluric acid.[2] The therapeutic level of salicylic acid in blood of a healthy adult is generally considered to be 150-300mg/l.[3] This level corresponds to urinary excretion ratio of salicylic acid to salicyluric acid of about 1:7.5.[1] Due to the availability of aspirin in numerous over-the-counter products and its widespread therapeutic use, the monitoring of its use or misuse is relevant. For this purpose, the use of antibodies recognising specifically the metabolite salicyluric acid, enables the development of non-invasive immunoassays based on the ratio of salicylic: salicyluric acid as indicator for monitoring aspirin concentration and to detect aspirin overdose in urine samples.

We report the development of a highly specific polyclonal antibody to salicyluric acid. This is of value for the development of non-invasive immunoassays using urine as sample, via calculation of the ratio salicylic acid: salicyluric acid for applications in therapeutic drug monitoring and toxicology settings.

Chemical structures



Salicylic acid



Salicyluric acid

Methodology

Materials and Method:

Salicyluric acid was coupled by way of a cross-linker to bovine thyroglobulin (BTG). The resulting immunogen was administered to adult sheep on a monthly basis to provide target-specific polyclonal antisera. IgG was extracted from the antisera and evaluated via competitive immunoassay. The purified antibodies were immobilized on a biochip platform (9mm x 9mm), which is also the vessel for the immunoreactions. The semi-automated analyser Evidence Investigator (EV3602, Randox Laboratories Ltd., Crumlin, Northern Ireland) was used.

Assay evaluation parameters:

Sensitivity: employing a competitive immunoassay format, salicyluric acid calibration curves were generated over 9 concentrations. %B/B0 values were calculated, where B is the signal measured for x µg/ml of analyte and B0 is the signal measured in the absence of analyte. The IC50 was calculated by taking 50% of signal from the zero calibrator and reading the value from the x-axis (standard concentration in µg/ml) in the calibration curve.

Specificity: specificity, expressed as % cross-reactivity (%CR) was calculated as follows:
%CR=[IC50 (salicyluric acid)/IC50 (cross-reactant)]x100

Precision: intra-assay precision was determined from the results of 6 replicates at different concentration levels within the same run. Results were expressed as %CV.

Sample analysis: 75 mg of aspirin were administered to two volunteers. Whole blood was analysed within 3 hours after intake, urine was analysed within 8 hours after intake.

Results

Initial evaluation results are reported.

Application to whole blood

Calibration range	Sensitivity IC50	Precision (n=6) %CV
0-1 µg/ml	0.021 µg/ml	≤12

10.050.481RDS

Application to urine

Calibration range	Sensitivity IC50	Precision (n=6) %CV
0-4 µg/ml	0.063 µg/ml	≤12

10.050.481RDS

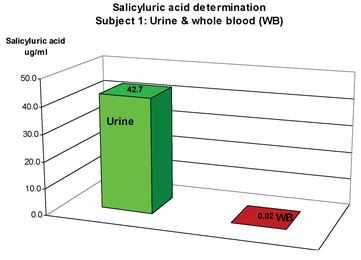
Specificity: whole blood and urine

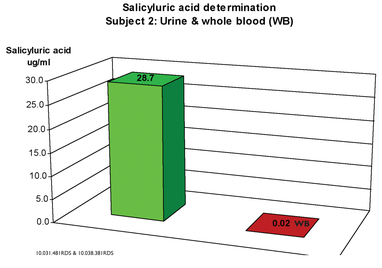
Compound	% Cross-reactivity
Salicyluric acid	100
Acetaminophen	<1
Acetylsalicylic acid	<1
Aspirin	<1
4-Aminosalicylic acid	<1
Benzoic acid	<1
Gentisic acid	<1
Gentisuric acid	<1
Hippuric acid	<1
Ibuprofen	<1
Methyl salicylate	<1
Salicylic acid	<1
Salsalate	<1

09.147.371RDS, 09.164.371RDS, 09.194.371RDS, 10.048.481RDS

Sample analysis

Intake: 75 mg of aspirin .
Blood samples were assessed within 3 hours after intake.
Urine samples were assessed within 8 hours after intake.





10.031.481RDS & 10.038.381RDS

As expected, salicyluric acid concentrations were higher in urine than in whole blood.

Conclusion

- The data indicates that this unique polyclonal antibody is highly specific for salicyluric acid.
- The antibody is sensitive and applicable to the assessment of both whole blood (sensitivity: 0.021 µg/ml) and urine samples (sensitivity: 0.063 µg/ml).

This polyclonal antibody is of value for the development of specific immunoassays for salicyluric acid. The antibody also has potential for non-invasive immunoassays based upon the ratio of salicylic: salicyluric acid as an indicator of overdose in urine samples.

References

1. Aspirin comprehensive prescribing information. Bayer data sheet for safety coated aspirin.
2. Patel, D.K., *et al.* Metabolism of aspirin after therapeutic and toxic doses. *Hum. Exp Toxicol.* 1990, 9:131-136
3. Goldkrank's Toxicologic Emergencies 2002, Ed: Goldfrank, Flomenbaum, Lewin, Howland, Hoffman, Nelson. 7th Edition. Published by McGraw-Hill.

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