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EFFECT OF AGE ON THE FUNCTIONAL GLYCOSIDASE ACTIVITY IN SMALL INTESTINAL MUCOSA OF PIGLETS

Introduction

Phytochemicals have recently regained interest in animal nutrition because of their potential health benefits. However, in nature these compounds often occur in their glycoconjugated forms, altering their bioavailability and physiological functionality. For example, two natural glycosides of quercetin, isoquercitrin and rutin, showed increased and decreased bioavailability, respectively, as compared to pure quercetin when administered orally to pigs¹. Moreover, glycoconjugation of drugs has been suggested as a method to protect pharmaceuticals from fast absorption and to deliver them to the distal parts of the gastro-intestinal tract (GIT)². After ingestion, glycosides may be hydrolysed into the active aglycon (i.e. phytochemical or drug) and sugar moiety by glycosidase enzymes present along the GIT. Therefore, characterization of the functional glycosides activity is essential to predict the fate of glycosides in the GIT of animals.

Aim

Evaluate the influence of age and small intestinal site on the functional activity of three glycosidases in the mucosa of piglets.

Experimental

Animals and treatments

Thirty-six median weight piglets, originating from six litters, were selected on farm and allocated to one of six sampling days (1 piglet per litter per time point):

- w-10 :10 days before weaning
 w :weaning (23 days of age)
 w+2 :2 days after weaning
- w+5 :5 days
- w+14 : 14 days
- w+28 : 28 days

scrapings were obtained from:

Sampling

- SI1 : 2.5 %
- SI2 : 25 % of small intestinal length

Piglets were humanely sacrificed and mucosal

SI3 : 75 %

Statistics

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Data were analysed by a general linear model with age, small intestinal site and their interaction as fixed factors

Analysis

Release of p-nitrophenol (pNP) from its respective pnitrophenyl-glycoside was measured in phosphate buffered extracts of mucosa to determine the functional activity of:

- a-gluc = a-glucosidase: exo- a-glucose
- β-gluc = β-glucosidase: hydrolysis of β-glucose
 β-gal = β-galactosidase: (1->4)-linked β-galactose

p _{age} < 0.010

p _{site} < 0.010

w+14 w+28

 $p_{age*site} = 0.294$

SI1

SI2

513

vith as With:

1 Unit [U] = 1 µmol of pNP released/min/g protein

β-galactosidase

Results



AGE effect: not significant

SITE effect: a-gluc activity in SI2 (1.6 U) significantly higher than in SI1 (1.2 U) and SI3 (1.4 U)

驒 α-gluc << β-gluc and β-gal:

- Pre-weaning: at least 15 and 20-fold, respectively
- Post-weaning: at least 5 and 10-fold, respectively



AGE effect: significant drop in β-gluc activity between:

- w-10 and w: -17.0 % (p=0.017)
- w and w+2: -55.2 % (p<0.010)
- <u>SITE effect</u>: β-gluc activity in SI3 (11.9 U) significantly lower than in SI1 (17.1 U) and SI2 (16.3 U)

Conclusion

- The small intestinal mucosa of piglets harbour <u>functional β-glucosidase and β-galactosidase activity</u> to hydrolyse phytochemical or drug glycosides prior to absorption and appearance in the blood.
- Activities are <u>higher in duodenum and proximal jejunum</u> as compared to distal jejunum and they are affected by the weaning event.
- On the contrary, functional <u>a-glucosidase activity</u> remained at <u>very low levels</u> across the small intestine for all ages, suggesting that a-glucosides might be more resistant to hydrolysis along the GIT of piglets.

Acknowledgement:

This work was supported by the Research Foundation Flanders (FWO) References:

¹Cermak, R., Landgraf S. and Wolffram S., The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. The Journal of nutrition, 2003. 133(9): p. 2802-2807.

² Friend, D., Colon-specific drug delivery. Advanced drug delivery reviews, 1991. 7(1): p. 149-199.

p age ≤ 0.010 40 p site ≤ 0.010 50 p age*site = 0.355 50 10 10

Age <u>Age AGE effect</u>: significant drop in β-gal activity between:

• w and w+2: -28.5 % (p<0.010)

w w+2 w+5

w-10

- w+2 and w+5: -28.5 % (p<0.010)
- SITE effect: β-gal activity in SI3 (19.7 U) significantly lower than in SI1 (25.8 U) and SI2 (24.9 U)

Perspectives

- Glycosides in nature appear most commonly as the β stereoisomer. The high β-gluc and β-gal activities indicate that they might be quickly hydrolysed and absorbed *in* vivo. Therefore, it would be opportune to design and synthesise α-glucosides for controlled and delayed release along the GIT.
- Here, only glycosidase activity of the mucosa is measured, but from our complementary study it appears that digesta also contain (lower) levels of these activities.





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