Impact of high dietary zinc concentrations on the horizontal antibiotic resistance gene transfer between enterobacteria in the intestine of gnotobiotic mice. (Einfluss hoher Zinkkonzentrationen im Futter auf den horizontalen Transfer von Antibiotikaresistenzgenen in Darm gnotobiotischer Mäuse). Lisa Rabes, S. Guenther, M. Doherr, L. H. Wieler, G. Loh* - Berlin/Potsdam

High concentrations of dietary zinc are widely used in piglets to reduce weaning-associated diarrhea and improve animal performance. However, a strong increase of intestinal *Escherichia coli* clones with multiple antibiotic resistances has been observed when piglets were fed a diet containing zinc at 2,500 ppm and it was proposed that zinc promotes horizontal gene transfer between gut bacteria (1). We aimed with our study to clarify in a highly-controlled gnotobiotic mouse model whether high concentrations of dietary zinc influence transfer of antibiotic resistance genes in the intestine.

Methods: Germ-free mice were fed a piglet diet based on wheat, barley, corn and soybean meal with zinc oxide at either 100 ppm or 1,900 ppm (atomic absorption spectrometry) and colonized with 10^8 cells of *E. coli* and *Klebsiella pneumoniae*, each. The latter strain served as a donor of an extended-spectrum β-lactamase-coding plasmid. Fecal material was collected every day over an experimental period of 20 days and colony forming units (cfu, $\log_{10}$) of *K. pneumoniae* (donor strain) and of *E. coli* without (recipient strain) and with (transconjugants) acquired antibiotic resistances were determined. Conjugation rates were calculated by dividing cfu of the transconjugant by cfu of the recipient strain. The area under the curve (AUC) approach was applied to integrate data obtained for each mouse, strain and sampling time point. Statistical analysis was performed with the Mann-Whitney U test.

Results: The bacterial strains successfully colonized the intestine of previously germ-free mice. Fecal bacterial numbers were highly variable. The minimal and maximal $\log_{10}$ of colony forming units and the integrated AUC values are given in table 1. Median values for the animals on the low zinc diet were $\log_{10}$ 9.9 (donor strain), $\log_{10}$ 8.7 (recipient strain) and $\log_{10}$ 6.0 (transconjugant). When the high zinc diet was fed, median values were $\log_{10}$ 10.0 (donor strain), $\log_{10}$ 8.5 (recipient strain) and $\log_{10}$ 5.6 (transconjugant). No effect of diet was observed the AUC of the donor and recipient values was compared. In contrast transconjugant AUC was significantly higher for the mice fed with 100 ppm of zinc ($P = 0.025$) indicating that zinc may inhibit the horizontal gene transfer. However, this assumption was not supported by the conjugation rate which did not differ between the feeding groups (data not shown).

Table 1: Colony forming units (log10) and AUC values of *K. pneumoniae* (donor) and of *E. coli* without (recipient) and with acquired antibiotic resistances (transconjugant) in mouse feces

Strain	low zinc minimal-maximal	high zinc minimal-maximal	low zinc median AUC	high zinc median AUC	p values
Donor	9.3 – 10.3	9.4 – 10.3	119.1	119.6	0.482
Recipient	6.4 – 9.6	7.3 – 9.6	104	101.9	0.225
Transconjugant	4.0 – 7.4	4.3 – 7.2	71.6	67.1	0.025

Conclusion: Our data does not provide a clear picture on possible effects of high dietary zinc on horizontal transfer of antibiotic resistance genes between intestinal bacteria because a significantly lower abundance of transconjugants was not supported by lower conjugation rates. The molecular mechanisms underlying the previously reported higher abundance of multi-resistant *E. coli* in piglets on a high-zinc diet require further investigation.

(1) BEDNORZ, C; OELGESCHLÄGER, K; KINNEMANN, B; HARTMANN, S; NEUMANN, K; PIEPER, R; BETHE, A; SEMMLER, T; TEDIN, K; SCHIERACK, P; WIELER, LH; GUENTHER, S; (2013): Int J Med Microbiol

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