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Topoisomerase poisoning by genistein in the intestine of rats

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HIGHLIGHTS

- Genistein (s.c.) significantly increases the amount of covalent topoisomerase IIα and β-DNA complexes *in the gut*.
- More persistent effects on covalent topoisomerase IIα and β-DNA complexes in the colon compared to the duodenum.

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ABSTRACT

The isoflavone genistein has been shown to act as topoisomerase II poison in various cell lines. Here, we address the question whether genistein is able to affect topoisomerase II *in vivo*. Juvenile male Wistar rats received either a single dose of genistein subcutaneously (s.c.; 10 mg/kg BW) or a lifelong isoflavone-rich diet encompassing *in utero*, lactation phase and 10 days of oral consumption, whereas genistein was mainly taken up as glycosides (25–50 mg/kg BW). The effects on the level of covalent topoisomerase II–DNA-complexes in the duodenum and colon were measured using the "Isolation of *in vivo* complexes of enzyme to DNA" (ICE)-bioassay. Simultaneously, serum as well as tissue concentrations of genistein and its metabolites were quantified by LC–MS.

Genistein (s.c.) significantly increased the amount of covalent topoisomerase IIα and β-DNA complexes in the gut, showing more persistent effects in the colon than in the duodenum. In case of a lifelong dietary isoflavone exposure, no effects on the stabilization of cleavage complexes was observed, except a slight increase of topoisomerase IIα–DNA-complexes in the colon. The differences between the exposure routes might be attributed to the higher serum concentration of the genistein aglycon after subcutaneous treatment probably due to circumvention of first-pass metabolism compared to oral consumption of an isoflavone-rich diet.

These data indicate that subcutaneously administrated genistein clearly possesses topoisomerase poisoning properties *in vivo*, whereas an isoflavone-rich diet containing genistein only caused a slight effect which relevance has to be clarified in further studies.

1. Introduction

The naturally occurring polyphenol genistein is one of the major isoflavones present in soy and soy-based products (Mazur, 1998; Price and Fenwick, 1985). High intake of isoflavones has been associated with beneficial effects on health including prevention of osteoporosis and cardiovascular diseases, alleviating menopausal disorders and is intensively discussed with respect to chemo-preventive properties against certain types of cancer (Adlercreutz, 2002; Andres et al., 2011). Epidemiological studies indicate a relationship between Asian diet high in isoflavones and a reduced incidence of breast and prostate cancer among the Asian population compared to Western countries (Adlercreutz, 2002; Magee and Rowland, 2004). Though the relationship between soy intake and health benefits seems to be very complex (Helferich et al., 2008), numerous dietary supplements based on isoflavone-

Abbreviations: BW, body weight; CTRL, control; GEN, genistein; ICE-bioassay, isolating *in vivo* complexes of enzyme to DNA-bioassay; IDD, isoflavone depleted diet; IRD, isoflavone rich diet; PND, postnatal day.

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enriched soy extracts are marketed especially for postmenopausal women, as these products are often used as an alternative to hormone replacement therapy (Wuttke et al., 2007). Additionally, infants may be exposed to high doses of isoflavones when fed with soy protein-based formulas (COT, 2003; Setchell et al., 1998). Against this background, concerns have recently been raised that high doses of genistein might also trigger adverse effects (Eisenbrand, 2007). Although the biological activity of genistein is often attributed to its affinity to the estrogen receptor, it also interferes with biochemical mechanisms such as apoptosis induction, regulation of cell growth and other signal pathways, mostly unrelated to its estrogenic activity (Klein and King, 2007; Taylor et al., 2009). So far, a spectrum of cellular targets has been reported to be affected by genistein, comprising its potency to act as a topoisomerase II poison *in vitro* (Bandele and Osheroff, 2007; Kalfalah et al., 2011). Topoisomerases are crucial cellular enzymes regulating the topological state of DNA and therefore, they are involved in fundamental DNA processes (Nitiss, 2009; Wang, 1996). As they generate transient DNA strand breaks during their catalytic cycle, they possess genotoxic potential representing important targets for widely used chemotherapeutic and cytotoxic drugs (Pommier, 2013). These topoisomerase poisoning drugs stabilize the intermediate of topoisomerase covalently linked to DNA converting the essential enzymes into cellular poisons (McClendon and Osheroff, 2007; Pommier et al., 2010). Recent studies suggest that topoisomerase poisons trigger chromosomal translocations, which might lead to the development of certain types of leukemia (Pendleton et al., 2014; Ross et al., 1996; Strick et al., 2000). Thus, the use of etoposide and doxorubicin in chemotherapeutic regimens has been associated with an increased risk of developing subsequent acute myeloid leukemia (Pendleton et al., 2014). Similarly, epidemiological data indicate that the risk of developing infant leukemia is increased approximately 10-fold by maternal consumption of foods high in naturally occurring topoisomerase II-poisons (Ross, 2000). Genistein has been characterized as topoisomerase II poison in various cell lines (Bandele and Osheroff, 2007; Kalfalah et al., 2011), but little is known to date about the occurrence of topoisomerase poisoning by genistein *in vivo*.

In the present study, we addressed the question whether genistein is able to stabilize the covalent intermediate of topoisomerase II and DNA in the intestine of Wistar rats, thus acting as topoisomerase poison *in vivo*. The tests were carried out using male rats, since possible estrogenic were of minor interest. The amount of cleavage complexes was investigated in the small intestine and colon, as these tissues are expected to yield high aglycon concentrations representing first sites of contact, while concomitantly having high expression levels of both topoisomerase II isoforms. Two different exposure patterns were chosen: One group of animals received an isoflavone-rich diet (IRD), being chronically exposed to genistein starting *in utero*, while the second group of animals was acutely exposed to genistein by subcutaneous application.

2. Material and methods

2.1. Chemicals

Daidzein (Dai) and genistein (Gen) were purchased from LC Laboratories (Woburn, MA, USA) and exhibit purities >99%. Dihydrodaidzein (DH-Dai) (>99%), daidzein-4′-β-D-glucuronide (Dai-4′-GlcA) (96%), daidzein-7-β-D-glucuronide (Dai-7-GlcA) (95%), daidzein-7-β-D-glucuronide-4′-sulfate (Dai-7-GlcA-4′-S) (98%), genistein-4′-β-D-glucuronide (Gen-4′-GlcA) (98%), genistein-7-β-D-glucuronide (Gen-7-GlcA) (98%), genistein-7-β-D-glucuronide-4′-sulfate (Gen-7-GlcA-4′-S) (97%) were purchased from Toronto Research Chemicals (North York, Canada). Dihydrogenistein (DH-Gen) (>99%) and rac-equol (>97%) were purchased from APIN Chemicals LTD (Abingdon, UK). *O*-Desmethylangolensin (ODMA) (>99%) and 6′-hydroxy-*O*-desmethylangolensin (6′OH-ODMA) (>99%) were purchased from Plantech UK (Reading, UK). Equol-4′-sulfate (Equol-4′-S) (>99%), equol-7-β-D-glucuronide (Equol-7-GlcA) (>99%) and [2,3,4,4-D_4]equol (D_4-Equol) were purchased from Santa Cruz Biotechnology (Dallas, USA). [2,3,4-$^{13}C_3$]Daidzein ($^{13}C_3$-Dai), [2,3,4-$^{13}C_3$]genistein-7-β-D-glucuronide ($^{13}C_3$-Gen-7-GlcA), [2,3,4-$^{13}C_3$]genistein ($^{13}C_3$-Gen) and [2,3,4-$^{13}C_3$]*O*-desmethylangolensin ($^{13}C_3$-ODMA) were provided by Nigel Botting (University of St. Andrews, UK).

Daidzein-4′-sulfate (Dai-4′-S) (98%), daidzein-7-sulfate (Dai-7-S) (99%), genistein-4′-sulfate (Gen-4′-S) (98%) and genistein-7-sulfate (Gen-7-S) (98%) were synthesized as recently described (Soukup et al., 2014).

All other chemicals and solvents used were of analytical grade. The standard stock and working solutions were prepared in DMSO.

2.2. Animal diets

The animals had free access to either an isoflavone depleted diet (IDD; Ssniff R/M-H Ssniff GmbH, Soest, Germany) or an isoflavone rich diet (IRD; Harlan Teklad 8604 rodent diet, Harlan–Winkelmann, Borchen, Germany) which were chosen based on former results (Hertrampf et al., 2009; Molzberger et al., 2013) and their compositions are depicted in Table 1. The protein sources in the Ssniff R/M-H diet are cereals and potatoes according to the manufacturers', while in the Harlan Teklad 8604 diet the proteins are derived from soy, fish meal and yeast. The high content of isoflavones in the IRD from Harlan–Winkelmann is derived from dehulled soybean meal and is specified in Table 2. Given the isoflavone content and daily food consumption (10–20 g/d/animal) and an average rat body weight at that age (BW) of 0.1 kg, the average oral intake resulted in a dose between 21.3 to 42.6 mg/kg BW daidzein and 24.8–49.5 mg/kg BW genistein per animal in the IRD group and around 1–2 mg daidzein or genistein/kg BW/day in the IDD group.

2.3. Animals

All animal handling and experimental conditions followed the "Institutional Animal Care and Use Committee guidelines", regulated by the German federal law for animal welfare.

Male and female Wistar rats were obtained from Janvier (Le Genest St Isle, France) and kept under controlled conditions of temperature (20 °C ± 1), relative humidity (50–80%) and illumination (12 h dark, 12 h light). Rats were mated and the dams were fed one of the two diets during pregnancy and nursing. These rats had *ad libitum* access to tap water and were fed their dedicated diet up to the age of 30 days. Fig. 1 gives an overview of the experimental design of the study. The present study was performed with male animals to exclude an overlay with estrogenic mechanisms.

Table 1
Composition of the different diets. IDD = isoflavone depleted diet (Ssniff). IRD = isoflavone rich diet (Harlan Teklad).

	IDD	IRD
Brutto energy (kcal/g)	4	3.93
Metabolizable energy (kcal/g)	3	3.3
Crude protein (%)	19.3	24.0
Crude fat (%)	3.3	4.0
Crude fiber (%)	4.4	4.5
N-free extractive (%)	55.1	46.64
C18:2 (Linols) (%)	1.49	1.87