Absorption and Excretion of Simmondsin after Different Administration Routes in Rats

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Adult rats received simmondsin by intragastric intubation (ig) or intraperitoneal injection (ip) or mixed in with food (MF). Food intake inhibition was noted after administration by any of these routes, starting within minutes after ig or MF administration and continuing for ±2 h in the former case and for 20 h in the latter. Following ip administration, food intake inhibition started after approximately 0.5 h and continued for about 2 h and was followed by a hyperphagic period. Simmondsin was measurable in the blood, but the concentration did not correlate with the anorexic effect. Only a fraction of the ingested or ip-administered simmondsin was excreted unmodified in the urine or feces. Ip administered simmondsin was detectable in the feces, indicating passage from the blood into the gut. The presence of simmondsin in the proximal gut seems to be necessary for the induction of anorexia.

Keywords: *Simmondsin; jojoba; absorption; excretion; rats*

INTRODUCTION

The jojoba plant (*Simmondsia chinensis*) is an evergreen shrub, native to the Sonoran desert of Mexico and the southern part of the United States. The seeds contain a liquid wax, used in cosmetics and as a lubricant (Wisniak, 1988). After extraction of the wax, the residue, jojoba meal, cannot be used as animal feed because of the presence of simmondsin [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucoside] and its derivatives (Elliger et al., 1973, 1974a,b; Van Boven et al., 1993, 1994a,b, 1995). Simmondsins were originally considered to be toxic because oral ingestion results in reduced food intake, weight loss, and, eventually, mortality (Booth et al., 1974; Verbiscar et al., 1980, 1981). However, other studies in rats have demonstrated that the food intake reduction induced by simmondsin can be reversed by the simultaneous application of the specific cholecystokinin receptor antagonist, devazepide (Cokelaere et al., 1995a). It has also been shown that defatted jojoba meal can be used to depress food intake in growing broiler breeder pullets (Arnouts et al., 1993) and induces satiation in rats (Cokelaere et al., 1995b), and it therefore has been suggested that simmondsin causes food intake reduction by inducing satiation via the cholecystokinin system (Cokelaere et al., 1995a).

Some authors have suggested that bacterial metabolites of simmondsin are absorbed into the bloodstream, since in contrast with orally administered simmondsin, intraperitoneally (ip) injected simmondsin apparently does not reduce 24 h food intake or body weight (Booth et al., 1974; Verbiscar et al., 1980). However, no attempt has been made to detect such metabolites in animals treated with simmondsin or defatted jojoba meal.

The four aims of the present study are to compare in rats the food intake reducing effect of simmondsin administered by various routes, to measure the concentration of simmondsin in the blood, to follow its elimination via the urine and feces, and to measure its *in vitro* degradation in rat feces.

EXPERIMENTAL DESIGN

Adult male Wistar rats (270−310 g) were housed under standard laboratory conditions (22 °C, relative humidity 40−60%, light from 8 a.m. to 8 p.m., free access to water and food). Complete rodent food (Carfil, Oud-Turnhout, Belgium; 18% crude protein) was given as meal in specially designed mangers to avoid spilling (Scholz, Overijse, Belgium). Pure simmondsin was prepared as described previously (Van Boven et al., 1993). Norit A Supra (activated charcoal) was obtained from Norit (Amersfoort, Netherlands).

Food Intake Tests. Thirty rats were divided into 3 groups ($n = 10$). On the day of the control and treatment measurements, all rats were deprived of food from 6 p.m. to 8 p.m. to synchronize the start of food intake. On the test day, 15 min before food presentation, group IG1 received simmondsin (S) by intragastral (ig) intubation [160 mg/kg of body weight (BW) in 1 mL of tap water], group IP1 received S by ip injection (160 mg/kg of BW in 0.5 mL of physiological saline), and group MF1 received S mixed in with the food (0.5% w/w). To control for any effect of the route of administration, group IG1 was also ip iinjected with 0.5 mL of physiological saline, group IP1 was also ig intubated with 1 mL of tap water, and group MF1 was also ip injected with 0.5 mL of physiological saline and ig intubated with 1 mL of tap water. All rats were adapted to the manipulations by injecting 0.5 mL of physiological saline and intubating them with 1 mL of tap water for 3 days before the start of the measurements. Food intake (FI) was measured 0.5, 1, 2, 3, 4, 8, 12, 16, and 20 h after food presentation by weighing the mangers and compared with the control food intake at similar moments after food presentation, measured the day before the administration of S. As control food intake

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was not different between the three groups (controlled by ANOVA), the values were pooled.

Simmondsin Blood Concentration Test. Two weeks after the first experiment, six rats chosen at random from each treatment group from the first experiment were used to monitor the concentration of S in the blood. FI and S blood concentrations were not evaluated in the same experiments, as the manipulations involved in blood sampling would have disturbed the FI pattern.

All rats had free access to water but were deprived of food for 24 h before the experiment to prevent any effect on simmondsin absorption or metabolism of food ingested the previous day. S was administered as described above for the IG1, IP1, and MF1 groups (IG2, IP2, and MF2 groups, respectively). IG2 and IP2 group rats were deprived of food for the whole test period, while the MF2 group had free access to S-containing food. Blood samples (0.5 mL) were taken, by tail clipping, 10 and 30 min and 1, 2, 4, 8, 12, 16, and 20 h after S administration and stored in heparinized vials at −20 °C until analyzed for S by HPLC (Van Boven et al., 1994c).

Elimination of Simmondsin via Feces and Urine. A further 18 rats, fasted for 24 h before the start of the test, were divided into 3 groups ($n = 6$) and received S as described in the first experiment (IG3, IP3, and MF3 groups, respectively). They were then placed in metabolic cages, designed to collect the urine and feces separately. All rats had free access to food and water during the measurement period. FI, urine, and feces production were measured 4, 8, 12, 16, 20, and 24 h after food presentation, and urine and feces samples were stored at −20 °C until analyzed for S. The concentration of S in the urine was measured using a modified HPLC method based on the method used to measure S levels in blood described previously (Van Boven et al., 1994c). One milliliter of an internal standard solution (phenyl β-glucopyranoside, 50 μg/mL) was added to 100 μL of sample. After extraction with 2 mL of activated charcoal in water (2%, Norit A), sonication, and centrifugation, samples were washed twice with water. S and the internal standard were eluted from the charcoal using acetone (4 mL). After sonication and centrifugation, the aliquots were filtered over a Dinaguard filter (50 μm) and evaporated under a nitrogen stream. The residue was redissolved in 200 μL of methanol/water (8/92 v/v). The original chromatographic method for blood samples was not suitable, as chromatograms of control urine samples showed interference with endogenous compounds at the retention times of S and the previously used internal standard (phenyl β-galactopyranoside). Replacing the C_{18} column by a C_8 column made it possible to separate S and the compound which coeluted on the C_{18} column; the internal standard, however, still showed interference but was successfully replaced by phenyl β-glucopyranoside. The Model L-6200 high-performance liquid chromatograph (Merck-Hitachi, Germany) used was equipped with an L-4250 UV−vis detector (Merck-Hitachi) operating at 217 nm. HPLC was carried out on a 60 RP-8 column using a methanol/water mixture (8/92 v/v) as eluent.

S levels in feces were measured after extraction of the feces sample with methanol containing 10 μg/mL of internal standard (phenyl β-glucopyranoside). The mixture was evaporated under a nitrogen stream, taken up in 1 mL of water, and extracted with 2 mL of activated charcoal (Norit A, 2%). The rest of the procedure was as described for urine samples.

Calibration graphs for both urine and feces samples were constructed by spiking urine and feces samples with known amounts of simmondsin (2, 5, 10, 20, 40, and 80 μg/mL of urine or feces) and 10 mg of internal standard. The ratios of the areas of simmondsin to the areas of the internal standard were plotted against the concentration of simmondsin. The curves were linear ($r = 0.998$ for urine and 0.992 for feces). The obtained chromatograms showed good separation between simmondsin and the internal standard without interferences from endogenous urine or feces components. The recoveries of simmondsin according to the described extraction procedure for concentrations of 5, 10, and 20 μg per milliliter of urine or gram of feces were quantitative for urine samples and 68% ± 7% for feces samples. The method allows for the determination of 0.5 μg of simmondsin/mL of urine and 2 μg of simmondsin/g of feces.

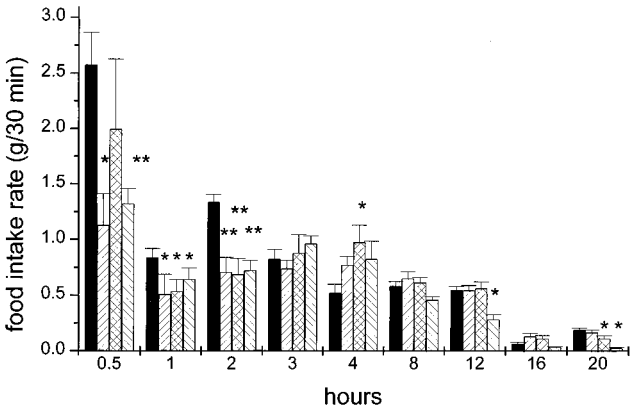

Figure 1. Food intake expressed as the amount eaten/30 min: (black bar) control food intake; (slashed bar) food intake after administration of 160 mg of simmondsin/kg of BW by ig intubation; (cross-hatched bar) food intake after administration of 160 mg of simmondsin/kg of BW by ip injection; (backslashed bar) food intake with simmondsin mixed in with the food (0.5% w/w); (*) statistically significant difference from control food intake ($p < 0.05$); (**) $p < 0.01$.

Metabolism of S in Feces. To measure any possible metabolism of S in feces, 10 mL of S (20 μg/mL of water) was mixed with 10 g of thoroughly ground fresh rat feces and incubated for 2 h at 37 °C with free air access. The concentration of S in 1 g was analyzed as described above, after 1 and 2 h.

The results were expressed as the mean ± SEM and analyzed by ANOVA (Microstat, Ecosoft, 1984) for statistical significance, with $p < 0.05$ taken as a significant difference.

RESULTS AND DISCUSSION

Experiment 1. FI, expressed as amount of food eaten per 30 min following administration of S by the different routes, is shown in Figure 1. Compared with control FI, FI was significantly decreased in the IG1 and MF1 groups from the first 30 min to 2 h after the start of measurements, after which time it returned to normal. For the MF1 group a decreased FI was also seen over the period between 4 and 20 h, but this was only statistically significant between 8 and 12 h and between 16 and 20 h. For the IP1 group, FI was significantly decreased between 0.5 and 2 h and between 16 and 20 h after food presentation but returned to normal for the rest of the measuring period or was even significantly increased between 3 and 4 h after food presentation.

As reported previously (Cokelaere et al., 1992), oral application of pure S, either by ig intubation or mixed in with the food, reduced FI in rats. This was also seen after ip injection, in contrast with the observations of other authors (Booth et al., 1974; Verbiscar et al., 1980) who found no FI reduction or body weight loss after ip injection of S in rodents. However, whereas administration by ig intubation or mixing S in with the food produced a statistically significant effect within minutes, a latency period was seen before the effect of ip injected S became evident. The most pronounced effect was seen during the first 2 h when the control rats eat at a fast rate, confirming our previous results (Cokelaere et al., 1992). The control FI of untreated rats decreased rapidly after satiation; thereafter, no difference was seen between control FI and FI of the IP1 and IG1 groups. In contrast, a period of hyperphagia (compared

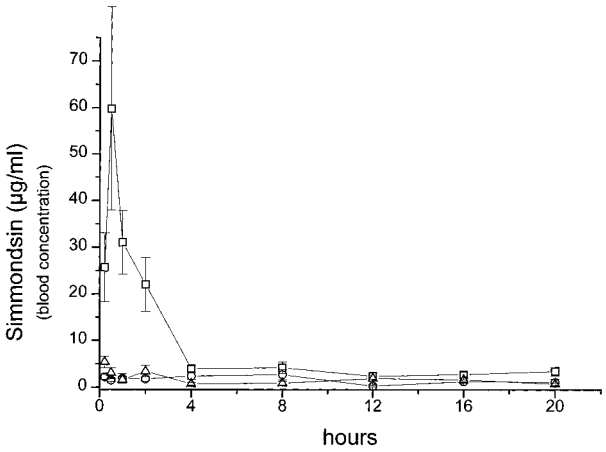

Figure 2. Changes in simmondsin blood levels after administration by different routes: (△) after administration of 160 mg of simmondsin/kg of BW by ig intubation; (□) after administration of 160 mg of simmondsin/kg of BW by ip injection; (○) with simmondsin mixed in with the food (0.5% w/w); mean ± SEM.

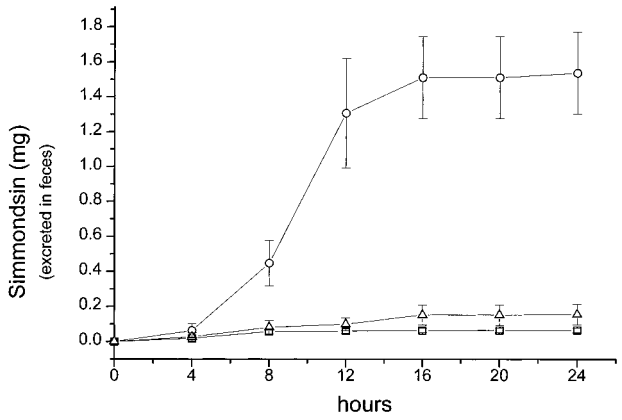

Figure 3. Cumulative excretion of unmodified simmondsin in the feces of rats treated with simmondsin: (△) after administration of 160 mg of simmondsin/kg of BW by ig intubation; (□) after administration of 160 mg of simmondsin/kg of BW by ip injection; (○) with simmondsin mixed in with the food (0.5% w/w); mean ± SEM.

with control FI) was seen in the IP1 group between 3 and 4 h after food presentation. This could explain why other authors (Booth et al., 1974; Verbiscar et al., 1980) did not note any effect on 24 h FI following ip administration of S in rodents. It appears that, after a short period of FI reduction, the rats make up the difference in FI to give a normal 24 h FI after ip administration. This observation, together with the fact that S administered by ig intubation or mixed in with the food exerts its effect almost immediately, enhances our suggestion that S itself is active and does not require prior metabolization by gut organisms (Booth et al., 1974; Verbiscar et al., 1980). However, our results cannot exclude the formation of active metabolites formed by acid hydrolysis in the stomach. Taste effects apparently had no major influence, since both the IP1 and IG1 groups showed a similar FI reduction to the MF1 group during the first hours after administration.

Bolus administration (160 mg/kg of BW) of S via ig intubation was not more effective during the first hours than the small quantities of S at low concentrations in the food. However, when the effect of ig administration was lost after 2 h, an effect continued to be seen for a further 16 h in the MF1 group. It seems that the presence of S in the stomach or small intestine is required to exert a FI inhibitory effect. After ig intubation, S is lost from the proximal gut within hours, as liquids are rapidly evacuated from an empty stomach (Cullen et al., 1993), explaining the loss of effect, while the effect remains in the MF1 group, as they continue to ingest S. These observations enhance the hypothesis that S exerts its FI inhibitory effect via cholecystokinin (CCK) liberation. Indeed, CCK-producing cells are located in the mucosa of the proximal gut and are normally stimulated by the intraluminal presence of peptides and fats (Morley et al., 1990). It can therefore be assumed that, if S acts by stimulating CCK release to produce its effect, it must be present in the proximal gut.

Experiment 2. Changes in blood levels of S in the IP2, IG2, and MF2 groups are shown in Figure 2. The total amount of S administered per rat by ip injection or ig intubation was approximately 46 mg, while the MF2 rats, who ate 10.03 ± 1.17 g during the first 20 h after food presentation, ingested about 50 mg of S. S was detectable in the blood 10 min after administration in all groups. When administered ip (IP2 group), the levels peaked at 59.8 ± 21.9 µg/mL after 30 min, fell rapidly to 3.99 ± 0.58 µg/mL after 4 h, and remained detectable at the basal level of 3.55 ± 0.38 µg/mL for up to 20 h after administration. In the IG2 and MF2 groups, S was detectable in the blood from 10 min to 20 h after administration at fairly constant low concentration (2.17 ± 0.45 and 1.63 ± 0.22 µg/mL, respectively).

S was absorbed from the gut into the bloodstream. The extent of absorption, however, did not correlate with the concentration in the gut, as the blood concentrations of S were almost identical in the MF2 and IG2 groups, but the gut concentration was much higher in the IG2 group than in the MF2 group, especially at the start of the experiment. This points to absorption mechanisms other than simple diffusion.

Although S was absorbed into the blood, the S concentration apparently did not correlate with its anorexic effect. Indeed, the S concentration in the blood was several times higher after ip injection (IP2) than after ig intubation (IG2) or administration via the food (MF2) during the first 4 h, but the anorexic effect was no greater in the IP1 group than in the IG1 or MF1 groups. In addition, although the blood levels of S were similar in all three groups from the fourth hour onward, FI reduction was not seen in this period in groups IG1 and IP1 but did occur in group MF1. These observations suggest that S is not active after absorption and raise the possibility that it exerts its anorexic effect only when present in the proximal gut.

Experiments 3 and 4. The extent of elimination of unmodified S in the feces was very low, being 0.07 ± 0.01, 1.54 ± 0.22, and 0.15 ± 0.06 mg for the IP3, MF3, and IG3 groups, respectively (Figure 3). As S is found in the feces of the IP3 group, one has to accept that S is excreted from the blood in the gut, either by simple back-diffusion or by biliary excretion, a feasible proposition, since the molecular weight of S (MW = 375) permits bile excretion in rats (Gibaldi, 1977). The latency period for the expression of full anorexia at the start of the feeding period in the IP1 group can possibly be explained by the fact that time is needed for S to be excreted from the blood into the gut. However, since rats have no gallbladder, the effect will become visible after only a small latency period. The anorexic effect of S in the IP1 was seen only during the period of high blood concentration in the IP2 group, probably because,

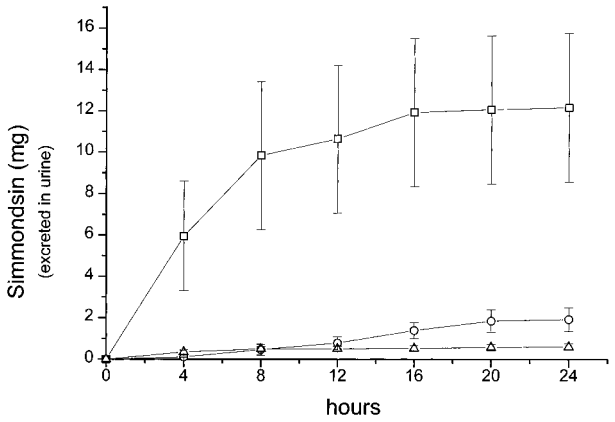

Figure 4. Cumulative excretion of unmodified simmondsin in the urine of rats treated with simmondsin: (△) after administration of 160 mg of simmondsin/kg of BW by ig intubation; (□) after administration of 160 mg of simmondsin/kg of BW by ip injection; (○) with simmondsin mixed within the food (0.5% w/w); mean ± SEM.

after this period, little additional S was excreted via the bile. This hypothesis, however, remains to be verified by measuring the content of S in the rats' bile.

Only traces of S were detected 1 h after *in vitro* incubation of 20 μg of S/g of feces, and no more S was detectable after 2 h of incubation. Because of the demonstrated fast fecal degradation of S, it can be assumed that most of the ingested S was metabolized in the gut. Indeed, no more than 1−2% of the original amount of S was recovered in the feces of the MF3 group. In the IG3 group, this was even lower. This confirms similar observations in sheep (Verbiscar et al., 1980). It can therefore also be assumed that elimination of S, after ip injection, via back-diffusion to the gut or via bile excretion, could be important, although the amount recovered in the feces in this group was low.

The elimination of unmodified S via the urine increased rapidly in the IP3 group to 9.85 ± 3.58 mg after 8 h and then increased slowly to 12.16 ± 3.60 mg after 24 h (Figure 4). About 26% of the total S injected was excreted by this route. The fate of the remaining 74% is unknown. However, as S was recovered in the feces of the IP3 rats, where it may be quickly metabolized, it seems probable that a major part of the ip injected S was also secreted in the feces. The total amount of S eliminated in the urine over 24 h in the MF3 and IG3 groups was minimal, being, respectively, 1.96 ± 0.56 and 0.61 ± 0.15 mg. The total amount of S ingested by the MF3 group was about 60 mg, as they ate 12.07 ± 0.76 g of food over 24 h. No S was detected in the organs or muscle tissue of sheep after long-term administration of S-containing defatted jojoba meal to sheep (Manos et al., 1986), so an accumulation in the tissues seems unlikely. We therefore assume that a major part of the S is metabolized in the gut, even after ip injection. Further investigations are needed to study the metabolism of simmondsin in rats.

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