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Grapefruit juice interaction with oral budesonide: equal effect on immediate-release and delayed-release formulations

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Grapefruit juice (GFJ) inhibits CYP3A activity in the gut wall, thereby decreasing first-pass metabolism of CYP3A substrates. In this study be evaluated the influence of GFJ on the systemic availability of budesonide, a CYP3A-metabolised drug, both from an extended-release (ER) formulation and plain capsules. Eight healthy men participated in this open crossover study. Three mg budesonide as ER capsules or plain capsules was swallowed with or without previous intake of GFJ. Regular-strength GFJ 200 ml was given three times a day for four days. Budesonide was administered immediately after the first intake on the fourth day. A simultaneous intravenous low dose of deuterium-labelled budesonide enabled estimation of bioavailability and absence of hepatic inhibition. Concentrations of labelled and unlabelled budesonide in plasma were measured. GFJ did not affect systemic clearance of budesonide. Although absorption of the ER formulation to a great extent occurs from ileum and proximal colon where CYP3A activity is lower than in the upper small intestine, GFJ about doubled bioavailability after both ER and plain capsules. In conclusion, regular intake of grapefruit juice doubled the bioavailability of both plain and delayed-release budesonide, probably because of inhibition of all mucosal CYP3A activity.

1. Introduction

It is now well accepted that the human gut mucosa may to a large extent contribute to the overall first-pass metabolism of drugs that are substrates for CYP3A, a subfamily of cytochrome P450 (Hebert et al. 1992; Kolars et al. 1994). This is mainly due to the catalytic activity of CYP3A4 in human enterocytes. However, the expression of CYP3A4 has been shown to decrease along the small intestine from duodenum to ileum and colon (McKinnon and McManus 1996; Thörn et al. 2005). With regard to the catalytic activity of CYP3A4 along the human gut, data are more scarce. In the small intestine, though, it has been demonstrated that CYP3A content and activity is highest in duodenum and proximal jejunum, and then declines to distal jejunum and ileum (Paine et al. 1997; Zhang et al. 1999).

Budesonide is a glucocorticosteroid, used clinically in the treatment of respiratory diseases, such as asthma and rhinitis, and in inflammatory bowel diseases like Crohn's disease. It is a CYP3A4 substrate (Jönsson et al. 1995), and recent findings (Seidegård et al. 2008) are in line with, and extend, the above data. In that study, a dose of only 16 mg ketoconazole was instilled by intubation at various locations of the gut just prior to instillation of 3 mg budesonide in solution. This caused an approximate two-fold increase in systemic availability from both jejunum and ileum, while no increase was noted from colon, thus indicating negligible catalytic activity of CYP3A4 in the colon. The systemic clearance, as measured by a low dose of deuterium-labelled budesonide, was unaffected by ketoconazole, demonstrating that the effect was due to inhibition of the intestinal, rather than the hepatic, metabolism.

The low activity of CYP3A in the colon is of great importance for drugs that are subject to substantial first-pass elimination in the gut wall, in that it opens up possibilities to increase systemic availability by formulating the drug in extended release (ER), as demonstrated by Gupta and co-workers (1999). They found a 53 % increased bioavailability of ER-formulated oxybutynin as compared with immediate-release (IR) oxybutynin. More recently, this concept was taken advantage of by Tubic-Grozdanis et al. (2008) who formulated simvastatin as a delayed-release tablet, thereby increasing its bioavailability three-fold compared with an immediate release capsule formulation. It was also suggested by these authors that a delayed formulation of a CYP3A4 substrate with high first-pass elimination in the upper intestine, would greatly diminish the risk for drug-drug and food-drug interactions.

An example of a food-drug interaction, with potentially serious consequences, is the increased bioavailability of several drugs that are CYP3A4 substrates, when administered after, or together with, grapefruit juice (GFJ), (Bailey et al. 1995; Ducharme et al. 1995; Kupferschmidt et al. 1995). The effect seems to be predominantly mediated by inhibition of CYP3A-activity in the intestinal mucosa, because when CYP3A4 substrates were given intravenously, they were not affected by GFJ ingestion (Ducharme et al. 1995; Kupferschmidt et al. 1995; Lown et al. 1997; Schmiedlin-Ren et al. 1997; Veronese et al. 2003). How-

ever, at amounts of GFJ much higher than normally ingested, the effect spills over into the liver, as shown by a prolonged half-life of midazolam, and a decreased ability to demethylate systemically administered erythromycin (Veronese et al. 2003). Using simvastatin as the substrate, it was shown that about 90% of the GFJ effect is lost within 24 h, and no effect could be seen 3 to 7 days after the last ingestion (Lilja et al. 2000). Similar results were achieved with midazolam after intake of GFJ, with an estimated recovery half-life of 23 h (Greenblatt et al. 2003).
Budesonide has been formulated as an extended-release (ER) dosage form for treatment of inflammatory bowel diseases. Between 45% and 70% of that formulation has been reported to be released in the ileum and ascending colon (Edsbäcker et al. 2003; Edsbäcker and Andersson 2004). The aims of the present study were (1) to document the anticipated interaction of GFJ with budesonide and (2) to find out whether the interaction would be less with the ER formulation than with an immediate release (plain) formulation. Between 45% and 70% of the Entocort dose has been reported to be released in the ileum and ascending colon (Edsbäcker et al. 2003; Edsbäcker and Andersson 2004).

2. Investigations and results

2.1. Dose administration

All oral doses were to be administered at 8 a.m. The maximal within-subject difference between visits was 30 min. The i.v. infusion always started one minute after administration of the oral dose. The average i.v. dose was 198 μg (range: 195–201) or 452 nmol (range: 443–458).

2.2. Pharmacokinetics

The mean plasma concentrations of deuterium-labelled budesonide after i.v. administrations are shown in Fig.1. The concentrations were similar for all administrations and there was no obvious effect of the GFJ. Individual estimated pharmacokinetic parameters of distribution and elimination of deuterium-labelled budesonide are shown in Table 1 after simultaneous administration of budesonide Entocort capsules without and with GFJ. In Table 2 the same i.v.-parameters are shown after co-administration of budesonide plain capsules without and with GFJ. Estimated pharmacokinetic parameters of distribution and elimination of deuterium-labelled budesonide with means and 95% confidence limits for treatments and treatment comparisons showed no statistically significant effect of GFJ intake on $t_{1/2}$, clearance, or V_d. Overall mean $t_{1/2}$ was

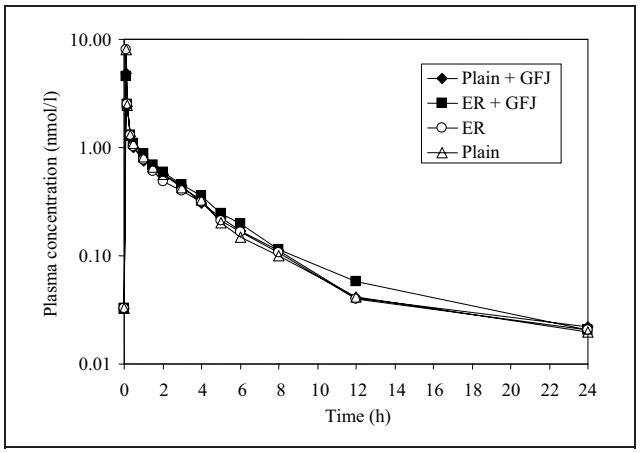

Fig. 1: Mean plasma concentrations of deuterium-labelled budesonide after i.v. administration

Table 1: Pharmacokinetic parameters of intravenously administered $^2H_8$-budesonide given simultaneously with budesonide ER capsules

Subject No.	$t_{1/2}$ (h)	AUC (h · nmol/l	CL (ml/min)	MRT (h)	V_d (l)	V_{ss} (l)
A. Without GFJ pretreatment						
101	2.8	4.38	1724	2.7	422	278
102	2.8	4.13	1838	3.9	452	425
103	2.6	3.32	2267	2.4	506	328
104	3.4	4.31	1716	3.3	512	335
105	3.5	5.34	1422	2.9	436	244
106	2.8	4.47	1694	2.7	407	273
107	2.7	3.29	2247	2.9	521	389
108	3.6	5.43	1379	4.0	428	329
Mean	**3.0**	**4.33**	**1786**	**3.1**	**461**	**325**
S.D.	**0.4**	**0.79**	**330**	**0.6**	**46**	**61**
Min	**2.6**	**3.29**	**1720**	**2.4**	**407**	**273**
Max	**3.6**	**5.43**	**2267**	**4.0**	**521**	**329**
B. GFJ pretreatment						
101	2.9	5.06	1506	3.6	378	322
102	4.1	4.78	1572	4.2	557	398
103	3.4	3.71	2035	3.1	595	381
104	3.0	3.57	2120	3.4	552	431
105	2.6	4.93	1532	2.7	341	253
106	4.4	6.69	1140	4.1	432	283
107	2.3	2.95	2533	2.8	508	420
108	3.3	5.14	1467	4.4	421	390
Mean	**3.3**	**4.61**	**1738**	**3.6**	**473**	**360**
S.D.	**0.7**	**1.17**	**451**	**0.7**	**93**	**66**
Min	**2.3**	**2.95**	**1140**	**2.7**	**341**	**253**
Max	**4.4**	**6.69**	**2533**	**4.4**	**595**	**431**

Table 2: Pharmacokinetic parameters of intravenously administered $^2H_8$-budesonide given simultaneously with budesonide plain capsules

Subject No.	$t_{1/2}$ (h)	AUC (h · nmol/l)	CL (ml/min)	MRT (h)	V_d (l)	V_{ss} (l)
A. Without GFJ pretreatment						
101	3.5	5.09	1490	3.0	457	266
102	4.1	4.29	1744	3.8	619	397
103	1.8	2.92	2548	2.3	397	346
104	4.8	4.70	1609	3.8	666	371
105	3.4	5.26	1428	2.7	421	230
106	3.2	6.61	1139	3.0	319	202
107	1.7	3.24	2334	2.3	347	329
108	3.1	3.80	1998	3.9	536	463
Mean	**3.2**	**4.49**	**1786**	**3.1**	**470**	**326**
S.D.	**1.0**	**1.20**	**477**	**0.7**	**126**	**88**
Min	**1.7**	**2.92**	**1139**	**2.3**	**319**	**202**
Max	**4.8**	**6.61**	**2548**	**3.9**	**666**	**463**
B. GFJ pretreatment						
101	2.5	4.61	1605	2.8	348	274
102	3.2	3.77	2011	3.7	550	449
103	3.1	3.27	2327	3.3	619	466
104	3.2	3.62	2084	3.6	572	445
105	3.6	5.52	1346	3.0	425	246
106	2.9	5.23	1432	3.2	357	275
107	4.8	3.61	2100	4.2	867	533
108	4.0	4.77	1565	4.5	538	420
Mean	**3.4**	**4.30**	**1809**	**3.6**	**535**	**389**
S.D.	**0.7**	**0.84**	**364**	**0.6**	**168**	**108**
Min	**2.5**	**3.27**	**1346**	**2.8**	**348**	**246**
Max	**4.8**	**5.52**	**2327**	**4.5**	**867**	**533**

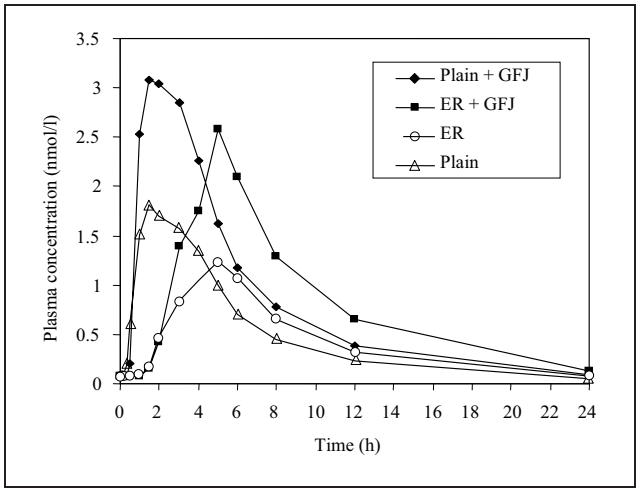

Fig. 2: Mean plasma concentrations of budesonide after oral administration

3.1 h without GFJ and 3.3 h with GFJ and the mean clearance was 1786 ml/min and 1774 ml/min, respectively. However, in this study MRT was found to be statistically significantly increased after intake of GFJ. The mean increase was 0.47 h (95% confidence limits: 0.17–0.76). Accordingly, V_{ss} was found to be statistically significantly increased after intake of GFJ with a mean increase of 15% (95% confidence limits: 6–24%).

The mean plasma concentrations of budesonide after the different oral administrations are shown in Fig. 2. Higher plasma concentrations were found after intake of GFJ, both for ER capsules and plain capsules. Terminal half-lives seemed to be slightly prolonged after oral administrations compared with i.v. administrations, probably because of prolonged absorption of budesonide.

Table 3: Pharmacokinetic parameters of budesonide after oral administration of budesonide ER capsules

Subject No.	$t_{1/2}$ (h)	AUC (h · nmol/l)	T_{max} (min)	C_{max} (nmol/l)	MAT (h)	F (%)
A. Without GFJ pretreatment						
101	3.0	7.75	180	1.37	3.8	11.5
102	4.1	10.23	480	1.10	6.7	16.2
103	5.4	10.65	180	1.47	5.3	20.8
104	3.9	5.41	300	0.73	5.3	8.0
105	3.3	8.15	300	1.24	5.0	10.0
106	6.1	13.39	300	1.53	7.4	19.5
107	3.0	3.58	300	0.60	5.0	6.9
108	5.4	10.66	240	1.52	4.8	12.7
Mean	**4.3**	**8.73**	**285**	**1.20**	**5.4**	**13.2**
S.D.	**1.2**	**3.17**	**95**	**0.36**	**1.2**	**5.2**
Min	**3.0**	**3.58**	**180**	**0.60**	**3.8**	**6.9**
Max	**6.1**	**13.39**	**480**	**1.53**	**7.4**	**20.8**
B. GFJ pretreatment						
101	4.1	22.41	240	3.31	4.0	29.1
102	5.0	22.46	360	2.55	6.0	30.4
103	4.7	12.94	300	1.68	6.9	22.7
104	5.8	12.32	300	1.57	6.1	22.5
105	4.7	18.88	300	2.58	6.0	24.9
106	5.7	41.29	300	5.94	6.3	40.6
107	4.1	11.69	180	1.49	4.5	25.5
108	4.0	18.26	300	2.58	4.2	23.1
Mean	**4.8**	**20.03**	**285**	**2.71**	**5.5**	**27.4**
S.D.	**0.7**	**9.62**	**53**	**1.45**	**1.1**	**6.1**
Min	**4.0**	**11.69**	**180**	**1.49**	**4.0**	**22.5**
Max	**5.8**	**41.29**	**360**	**5.94**	**6.9**	**40.6**

Table 4: Pharmacokinetic parameters of budesonide after oral administration of budesonide plain capsules

Subject	$t_{1/2}$ (h)	AUC (h · nmol/l)	T_{max} (min)	C_{max} (nmol/l)	MAT (h)	F (%)
A. Without GFJ pretreatment						
101	6.0	13.37	90	2.46	3.6	17.2
102	5.3	10.97	60	1.82	2.4	16.5
103	7.1	10.53	240	1.33	6.3	23.1
104	3.6	9.96	90	1.83	1.3	13.8
105	4.7	10.17	240	1.44	4.9	12.5
106	5.3	25.74	90	5.57	3.0	25.3
107	4.0	5.74	120	1.36	2.7	11.6
108	2.9	8.08	300	1.43	3.0	13.9
Mean	**4.9**	**11.82**	**154**	**2.16**	**3.4**	**16.7**
S.D.	**1.4**	**6.04**	**91**	**1.43**	**1.5**	**5.0**
Min	**2.9**	**5.74**	**60**	**1.33**	**1.3**	**11.6**
Max	**7.1**	**25.74**	**300**	**5.57**	**6.3**	**25.3**
B. GFJ pretreatment						
101	5.2	18.23	60	3.68	3.4	25.2
102	6.0	20.62	90	3.53	3.3	35.7
103	5.3	14.04	180	2.03	4.9	28.2
104	5.0	14.29	240	2.00	4.5	25.7
105	4.2	32.27	90	6.93	2.1	37.5
106	5.5	36.12	180	5.94	3.7	44.6
107	3.7	8.32	60	2.27	0.6	15.0
108	4.9	17.24	60	3.50	1.9	23.3
Mean	**5.0**	**20.14**	**120**	**3.74**	**3.0**	**29.4**
S.D.	**0.7**	**9.46**	**70**	**1.82**	**1.4**	**9.4**
Min	**3.7**	**8.32**	**60**	**2.00**	**0.6**	**15.0**
Max	**6.0**	**36.12**	**240**	**6.93**	**4.9**	**44.6**

Individual estimated pharmacokinetic parameters of absorption of budesonide after administration of budesonide ER are shown in Table 3 without and with GFJ administration. In Table 4 the same parameters are shown after administration of budesonide plain without and with GFJ administration. Terminal half-lives seemed to be slightly prolonged after oral administrations compared with i.v. administrations, probably because of prolonged absorption of budesonide. A summary with means and 95% confidence limits for treatments and treatment comparisons is given in Table 5. The mean systemic availability was statistically significantly increased after intake of GFJ, both for ER and plain capsules. The mean increase was 94% (95% confidence limits: 68–126). There was no statistically significant difference in the increase of bioavailability between ER and plain capsules. Similar results were obtained for C_{max}. Nor was there any statistically significant effect of GFJ intake on $t_{1/2}$ or MAT.

Intake of GFJ seemed to increase the bioavailability of budesonide similarly for ER and plain capsules.

3. Discussion

GFJ is known to interact with drugs that are metabolised in the intestinal gut mucosa by CYP3A (Bailey et al. 1995; Ducharme et al. 1995; Kupferschmidt et al. 1995; Lown et al. 1997). Since the expression and activity of this enzyme has been shown to be very low in the colon (McKinnon and McManus 1996; Thörn et al., 2005; Seidegård et al. 2008), it was to be expected that the GFJ effect on bioavailability would be less for an ER formulation of a CYP3A4 substrate than for an immediate formulation (Tubic-Grozdanis et al. 2008).

The aim of the present study was to investigate the impact of GFJ ingestion on the bioavailability of budesonide administered as two different formulations: a plain capsule with a

Table 5: Mean pharmacokinetic parameters and contrast of systemic uptake of orally administered budesonide

		without GFJ		GFJ		GFJ vs without GFJ[1]	
Parameter	Treatment	mean	95% conf.lim.	mean	95% conf.lim.	mean	95% conf.lim.
$t_{1/2}$ (h)	**ER**	4.3	3.57–4.76	4.8	4.09–5.45	115	93–140
	Plain	4.9	4.08–5.44	5.0	4.25–5.67	104	85–128
	ER vs plain	88	71–107	96	79–118	110	82–147
AUC (h · nmol/l)	**ER**	8.73	6.94–9.52	20.03	15.75–21.58	227	181–283
	Plain	11.82	9.24–12.66	20.14	15.64–21.43	169	135–212
	ER vs plain	75	60–94	101	81–126	134	98–184
C_{max} (nmol/l)	**ER**	1.20	0.93–1.40	2.71	2.00–3.01	216	161–288
	Plain	2.16	1.55–2.33	3.74	2.76–4.15	178	133–238
	ER vs plain	60	45–80	72	54–97	121	80–182
MAT (h)[1]	**ER**	5.42	4.59–6.25	5.49	4.67–6.32	0.07	−1.10–1.24
	Plain	3.40	2.58–4.23	3.03	2.21–3.86	−0.37	−1.54–0.80
	ER vs plain	2.02	0.85–3.19	2.46	1.29–3.63	0.44	−1.21–2.10
F (%)	**ER**	13.2	10.6–14.3	27.4	23.1–31.1	218	176–269
	Plain	16.7	13.39–18.7	29.4	24.2–32.5	174	141–214
	ER vs plain	76	62–94	96	78–118	125	93–169

[1] Contrast for MAT are differences, otherwise ratios are given in %.

release predominantly in the proximal small intestine, and ER capsule with a major release in the distal small intestine and proximal colon (Edsbäcker et al. 2003; Edsbäcker and Andersson 2004). Deuterium-labelled budesonide was given simultaneously with the oral formulations to measure systemic clearance, and to allow calculations of bioavailability. It has been proposed that budesonide, despite its low oral availability, is an intermediate clearance drug with regard to its hepatic clearance (Seidegård et al. 2008). Thus, a spill-over of the GFJ inhibition from the gut to the liver as noted after high doses of GFJ (Veronese et al. 2003) should be readily observable as a decreased clearance of deuterium-labelled budesonide. This was not seen in the present study.

The bioavailability of budesonide increased statistically significantly two-fold with both formulations after GFJ ingestion. The mean increase was slightly, but not significantly, greater after budesonide ER capsule administration. Similar effects were seen on C_{max}.

As suggested by Tubic-Grozdanis and coworkers (2008), one would have expected a larger effect of GFJ on plain budesonide than on ER, particularly in the light of results obtained in a recent study in which budesonide was given at different intestinal levels (Seidegård et al. 2008). In that study, it was noted that the systemic availability of budesonide administered in the colon did not increase after pre-administration of ketoconazole, indicating lack of CYP3A activity in the colon.

A likely explanation to the similar effect of GFJ on plain and ER budesonide is the design of the present study. The intake of GFJ regularly during several days probably inhibited CYP3A4 activity in the whole intestine, implying that not only the colon but also the small intestine were devoid of such metabolic capacity. As a consequence, no difference in the interactive effect of GFJ on the studied dosage forms could be anticipated. This is an important finding, as in practice patients are either not drinking GFJ or they have it on an everyday basis. One must remember, however, that even if plain and ER budesonide together with GFJ are taken up to the same extent, there exists an interaction that may call for dose adjustment.

In summary, regular intake of grapefruit juice doubled the bioavailability of both plain budesonide and delayed-release budesonide. Therefore, releasing budesonide more distally in the gut does not reduce the interaction potential with everyday grapefruit juice.

4. Experimental

4.1. Study design

The study was open, randomised and of a crossover design. It included four different treatment periods (see below). In all treatments, an intravenous dose of 0.2 mg deuterium-labelled budesonide was given at the same time as orally given budesonide. The total study time was between 8 to 12 weeks. There was also a follow-up visit after approximately 1 week.

4.2. Study drugs

Extended-release formulation of budesonide, Entocort capsules, 3 mg, were manufactured by AstraZeneca Pharmaceutical Production in Södertälje, Sweden. Plain budesonide, 3 mg in a capsule, was manufactured at the Department of Pharmacy, AstraZeneca R&D Lund, Sweden.

An intravenous solution of deuterium-labelled ($^2H_8$) budesonide (0.025 mg/ml) was manufactured at the Department of Pharmacy, AstraZeneca R&D Lund, Sweden.

Fresh grapefruits were squeezed, and the juice was mixed and dispensed in batches of 200 ml at AstraZeneca R&D Lund and quickly frozen.

4.3. Subjects

Ten subjects were enrolled into the study. Eight subjects (Nos 101–108) were randomised and completed the study. All subjects were healthy male Caucasians. None was a user of tobacco, or nicotine in any form, such as gum or patch. Two subjects were past smokers, more than four years before study start. Mean age of the eight subjects was 28 years (range 20–42 years), mean weight 75 kg (60–91 kg), and mean height 185 cm (175–200 cm). No subject had a previous or current medical condition, and none was currently using any medication. The study was carried out in accordance with the Declaration of Helsinki, and was approved by the Ethics Committee at the University Hospital in Lund, Sweden. Written informed consent was obtained from each subject.

4.4. Administrations

All subjects were studied during a total of four different treatment periods. The drug administrations were performed in the morning at 8 a.m.

4.5. Oral treatments

Washout intervals between treatment periods were at least three days.

Treatment I: The subjects were instructed to arrive at the clinic at 6 a.m., having fasted (no solid or viscous food) since 10 p.m. the previous day. Two indwelling catheters were inserted into brachial veins, one in each forearm. Budesonide ER capsule, 3 mg (7.0 μmol), was given as a single oral dose.

Treatment II: GFJ (200 ml) was given three times a day; at breakfast, lunch, and supper during 3 days. On the fourth day GFJ was given just before administration of budesonide ER capsule as in Treatment I.

Treatment III: Similar to that of Treatment I except that plain budesonide, 3 mg (7.0 μmol), was given as a single oral dose.

Treatment IV: GFJ (200 ml) was given as in Treatment II for 3 days. On the fourth day GFJ and plain budesonide were given at the same time as in Treatment II.

4.6. Intravenous infusion

In all treatments, starting at the same time as the orally given budesonide, an intravenous dose of 0.2 mg (0.46 μmol) deuterium-labelled budesonide was given over 5 min. Thereafter the intravenous (i.v.) line was flushed with 1 ml of saline. The syringe used for i.v. administrations was weighed before and after drug administrations to ascertain an accurate dose determination.

4.7. Blood sampling

Na-heparinised blood samples (7 ml) were obtained from an indwelling cannula inserted into an arm vein not used for drug infusion, before (0 min) and at 5, 10, 20, 30, 60, 90 min, and at 2, 3, 4, 5, 6, 8, 12, 24 h after administration of budesonide. At each sampling, the first millilitre of blood was discarded and, after collection of the sample, the catheter was flushed with 2 ml heparinised saline (10 IU/ml) to keep it patent. The blood samples were centrifuged at 1300 × g for 10 min. Plasma was transferred to polypropylene tubes, which were stored at −20 °C until analysed for budesonide and deuterium-labelled budesonide.

4.8. Assays

Budesonide in plasma was assayed by a liquid chromatography mass spectrometry method. The lower limit of quantification (LOQ) was 0.033–0075 nmol/l depending on the different plasma volumes (Kronkvist et al. 1998).

4.9. Pharmacokinetic evaluation

In all calculations the density of the i.v. solution was calculated as 0.989 g/ml and the budesonide concentration as 25 μg/ml. The oral doses were considered to be 3.0 mg. The molecular weight of budesonide and deuterium-labelled budesonide is 431 g/mol and 439 g/mol, respectively. The terminal elimination rates of budesonide and deuterium-labelled budesonide, $k_{\lambda z}$, were estimated for each subject and treatment period.

For calculation of pharmacokinetic parameters and plotting of individual concentration curves concentrations below LOQ were excluded or estimated as follows:

- The concentration at time zero was estimated at zero.
- All samples after the last concentration above LOQ were excluded.
- A sequence of non-quantified values before the first quantified value were estimated at zero except for the last one which was estimated at LOQ/2 (the LOQ for this sample was used).
- When plotting mean concentration curves, individual values below LOQ after the last value above LOQ were estimated using exponential extrapolation.

Maximum plasma concentration, C_{max}, and time when it occurred, T_{max}, were located. The non-compartmental pharmacokinetic parameters AUC, $t_{1/2}$, CL, MRT, V_d and V_{ss} were calculated by standard procedures. Actual sampling times were used. The calculation of systemic availability (F) and mean absorption time (MAT) after oral administrations assumed identical pharmacokinetics of deuterium-labelled and unlabelled budesonide. F was calculated as CL×AUC/Dose, CL being estimated from the simultaneous i.v. administration of deuterium-labelled budesonide. MAT was estimated as the difference between AUMC/AUC for the oral administration and MRT for the concomitant i.v. administration of deuterium-labelled budesonide.

4.10. Statistical analysis

Clearance of deuterium-labelled budesonide was estimated and compared between the treatments using a multiplicative analysis of variance model with subject and treatment as fixed factors. The effect of GFJ on the systemic metabolism of budesonide was described by comparing the treatments with and without GFJ.

The systemic availability of budesonide was estimated and compared between the four different oral treatments using the same model as above. The overall effect of GFJ intake was estimated as well as the effects on budesonide ER and plain budesonide administered separately. The GFJ effects on the two formulations were also compared. All relative and absolute bioavailabilities were described with geometric mean and 95% confidence limits.

Other pharmacokinetic parameters were estimated and compared between the treatments using analysis of variance models with subject and treatment as fixed factors. Multiplicative models (and geometric means) were used for $t_{1/2}$, V_d, V_{ss}, AUC, and C_{max}, additive models (and arithmetic means) were used for MRT and MAT.

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