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Phase I clinical study to select a novel oral formulation for ibandronate containing the excipient sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC)

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The aim of this study was to select a novel oral formulation for ibandronate (IBN, CAS number: 138926-19). In four cohorts of 28, 21, 19 and 29 healthy volunteers, the impact of the carrier molecule sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC, CAS number: 203787-91-1) on the bioavailability of IBN was investigated. Within each cohort different oral formulations with one dose of ibandronate (30 mg) and three different ratios of IBN:SNAC (1:5, 1:10 and 1:20) were compared to the approved oral IBN tablet formulations (150 and 50 mg IBN) in a 4-way cross-over design and a one week washout between the administrations. The highest mean IBN exposure was achieved with a capsule formulation containing drug-coated beadlets and an IBN:SNAC ratio of 1:5. AUClast and Cmax of IBN were approximately 1.3- and 2.2-fold higher compared to the reference treatment (150 mg IBN without SNAC). Increasing the post-dose fasting duration from 15 to 30 min resulted in a more than 2-fold increase in AUClast, while superimposable IBN serum concentration-time profiles were achieved after a 30 and 60 min fast. The tolerability of the IBN/SNAC treatments in all cohorts was similar to that in the IBN reference groups and most adverse events (AEs) were of mild to moderate intensity.

1. Introduction

When combined with another charged molecule (e.g. heparin), the pharmaceutical excipient sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC, CAS number: 203787-91-1) has been shown to facilitate the transport of that molecule across the gastrointestinal tract (Leone-Bay et al. 1998). In the current investigation, SNAC has been co-formulated with ibandronate (IBN, CAS number: 138926-19), a potent, nitrogen-containing bisphosphonate that has been shown to induce significant increases in bone mineral density (BMD) at the lumbar spine and the hip and decrease the risk of osteoporotic fractures in postmenopausal women (Delmas et al. 2002; Chesnut et al. 2004; Miller et al. 2005; Reginster et al. 2006) and is approved for the treatment and prevention of postmenopausal osteoporosis and for the treatment of metastatic bone disease.

Ibandronate exhibits a low and highly variable oral bioavailability ($\sim 0.6\%$). As its oral absorption is reduced by concomitant food intake (Barrett et al. 2004), the bisphosphonate must be taken under fasting conditions and fasting must continue for at least 60 min (Bonviva® 150 mg tablets) or 30 min (Bondronat® 50 mg tablets) post-dose to ensure adequate absorption and efficacy. These food intake restrictions may result in inconvenience for some patients, which might affect long-term compliance. Co-formulation of SNAC with IBN is expected to result in a faster absorption of IBN from the gastrointestinal tract, which might allow for a reduction of the post-dose fasting duration.

The primary objective of the study was to select an IBN/SNAC formulation type as well as the appropriate IBN:SNAC ratio required to increase the oral bioavailability of the bisphosphonate. A parallel cohort design (cohorts I to III) was selected to compare different formulation types to permit a full assessment of safety, i.e. maximizing the number of subjects exposed to better characterize the safety profile of the IBN/SNAC combination. Within each cohort, a cross-over design was chosen to compare the impact of the IBN:SNAC ratio on the absorption of IBN for a formulation type. The selected formulation type and IBN:SNAC ratio from cohorts I to III was further evaluated with respect to the impact of the post-dose fasting duration on the exposure to IBN in a sequential cohort IV.

2. Investigations and results

2.1. Demographics

The majority of subjects enrolled were male. The percentage of females was 21%, 10%, 32% and 45% in cohorts I, II, III and IV, respectively. More than 90% of subjects were Caucasian. The age of the subjects varied between 18 and 60 years with mean values of 31.6, 24.2, 24.7 and 36.5 years in cohorts I to IV. Body weight, height and body mass index were comparable between the cohorts, ranging from 52 to 89.5 kg, 156 to 193 cm, and 18.2 to 33.3 kg/m^2, respectively.

2.2. Safety

The majority of the adverse events were mild to moderate in intensity. No adverse event was of serious intensity. There was no increase in the incidence of adverse events with increasing doses of SNAC. The most common adverse events across all four cohorts were musculoskeletal and connective tissue disorders (mainly back pain), gastrointestinal disorders (mainly nausea), and nervous system disorders (mainly headache). There were no clinically significant changes in vital signs and ECG parameters.

2.3. IBN and SNAC dissolution from formulation

As shown in Fig. 1, tablet formulations 1 and 2 showed comparable sigmoidal dissolution profiles for SNAC and IBN, suggesting co-dissolution of the ingredients. A lag-time of approximately 30 min was observed prior to IBN release from the formulation. The capsules containing drug-coated beadlets (formulation 3) or the spray-granulate (formulation 4) did exhibit an early release of ibandronate, with a slight lag in release of SNAC. The spray granulate did show a first order release pattern for IBN and a zero-order release profile for SNAC, while the beadlet formulation resulted in a zero-order release for both IBN and SNAC, an indication for co-dissolution.

2.4. NIR Chemical Imaging

For formulations 3 and 4, spectra extracted from each sample image were projected onto the PLS model and score images for IBN, SNAC and PVC were calculated. Fig. 2 shows the SNAC images in formulation 2 (drug-coated beadlets) and formulation 4 (spray-granulate) along with the histogram plots of the score values and statistical parameters. The pixel score value corresponds to the concentration of SNAC in the sample matrix. The darker pixels symbolize higher localized concentration of SNAC. Results demonstrated that SNAC is more uniformly distributed around IBN in the beadlet formulation than in the granulate. In addition, the lower standard deviation and smaller kurtosis value in the beadlets sample image indicate lower variability, thus a higher degree of homogeneous SNAC distribution within the sample matrix.
Results obtained from PLS score images of SNAC were further verified by the RGB images constructed using the individual score images of IBN, SNAC, and PVP, as shown in Fig. 3. RGB images revealed that SNAC is more homogenously mixed with IBN in the beadlet formulation, whereas evident agglomeration of SNAC and IBN was observed in the granule sample. In the beadlet formulation, IBN particles alone were difficult to be spatially resolved under the 20 μm/pixel objective of the image system, which may be an indication for a uniform and close contact between SNAC and IBN, prohibiting self-aggregation of IBN. Tables 1–4

2.5. Pharmacokinetics

The pharmacokinetics of IBN were studied in each cohort. Mean serum concentration-time plots of IBN by treatment for each cohort are shown in Fig. 4. Mean (± SD) PK parameters by treatment for each cohort are summarized in Tables 1–4. Estimated ratios for AUClast and Cmax with respect to treatment by cohort are shown in Table 5.
In cohorts I and II where two different types of SNAC containing tablet formulations (formulations 1 and 2) containing 30 mg IBN each were compared to the approved 150 mg IBN tablet formulation, the exposure to IBN expressed as AUClast was the highest with reference formulation. In cohort I, this higher exposure with the reference formulation was statistically significant compared to all IBN/SNAC ratios, while in cohort II the advantage with the reference formulation was statistically significant compared to the IBN:SNAC ratios of 1:5 and 1:20 only. In both cohorts, AUClast decreased in the following order: reference 150 mg formulation > IBN:SNAC 1:10 ratio > IBN:SNAC 1:5 ratio > IBN:SNAC 1:20 ratio. The mean AUClast for the 150 mg reference formulation was approximately 2.2- and 1.6-fold higher than for the IBN:SNAC formulation with the 1:10 ratio in cohorts I and II, respectively.
The highest overall exposure to IBN was achieved with the SNAC containing formulation type 3 tested in cohort III (drug-coated beadlets). AUClast of IBN decreased in the following order: IBN:SNAC 1:5 ratio > IBN:SNAC 1:10 ratio > IBN:SNAC 1:20 ratio > reference 150 mg formulation. AUClast and Cmax of IBN with an IBN:SNAC 1:5 ratio were approximately 1.3- and 2.2-fold higher than with the reference 150 mg treatment. This difference was statistically significant for Cmax only.
The coated beadlets formulation with an IBN:SNAC ratio of 1:5 was selected for a food effect assessment in cohort IV. Compared to a post-dose fasting duration of 15 min as studied in cohort III, an increase of the fasting time to 30 and 60 min did result in a 2.3- and 2.5-fold increase in AUClast and a 1.7- and 1.8-fold increase in Cmax of IBN, respectively. IBN serum concentration-time profiles after a 30 and 60 min fast were superimposable.
As a protocol amendment, in cohort IV, an additional formulation type 4 (granulate formulation administered in hard gelatin capsules, IBN:SNAC ratio 1:5, post-dose fast of 60 min) was investigated. Results demonstrated that the mean AUClast and Cmax after a 60 min fast with the granulate formulation were approximately 4.6- and 4.2-fold lower, respectively, as compared to the beadlet formulation with a 30 or 60 min post-dose fast. This difference was statistically significant.
Median Tmax with all SNAC containing formulations was higher compared to Tmax of the reference formulations (average 0.33 h vs. 0.667 h).

3. Discussion

Absorption of a substance following ingestion is determined largely by its physicochemical properties such as molecular weight, solubility pKa and lipophilicity (Song et al. 2004). Thus, the poor absorption of bisphosphonates is proposed to be due to their polarity, being negatively charged at physiological pH. This polarity hinders transcellular transport through epithelial membranes, and paracellular transport is thought the likely route of absorption (Lin 1996). As for other bisphosphonates, it is expected that IBN absorption occurs high in the gastro-intestinal tract (Brayden et al. 1997; Mitchell et al. 1998). This assumption is supported by the rapid absorption within 1 hour, as well as by the influence of gastric acidity and the presence of food on the extent of absorption (Barrett et al. 2004). Consequently, an early release from the formulation together with a protection from degradation in the gastric environment is necessary for increasing the absorption of IBN.
The carrier molecule SNAC, when combined with a target substance, has been shown to form a weak, non-covalent complex that enables absorption of the target substance while preserving its chemical integrity (Brayden et al. 1997; Wu and Robinson 1999a, b; Leone-Bay et al. 2001; Malkov et al. 2002). SNAC and related carriers enable absorption primarily via the transcellular pathway, without compromising the integrity of the intestinal epithelium (Ding et al. 2004).
This clinical investigation was undertaken to select the formulation type that results in the highest systemic exposure to IBN

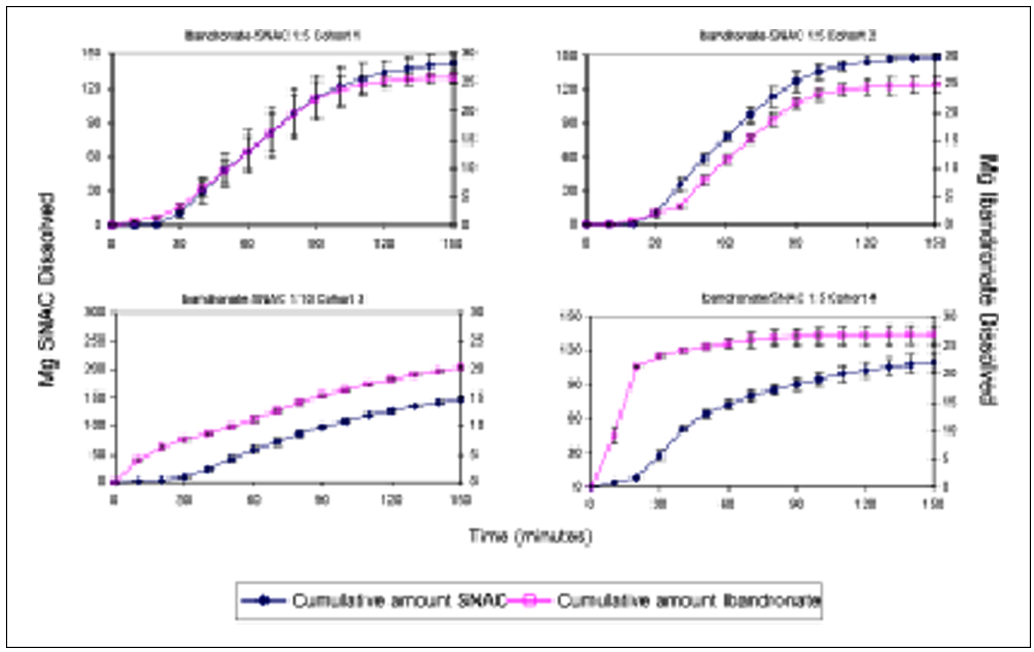

Fig. 1: Dissolution profiles of ibandronate and SNAC from different formulations

in the presence of SNAC. The underlying assumption was that intimate contact between the bisphosphonate and the carrier at the site of absorption would be required. This hypothesis is based on a study in beagle dogs. In a cross-over study which used 4 dogs, heparin and SNAC were either co-formulated or administered alone but concomitantly in two different soft gel capsules. The results showed that heparin absorption occurred only when heparin was formulated (or processed) along with SNAC (Emisphere unpublished data).

In the current trial, the drug-coated beadlet formulation (formulation 3) tested in cohort III resulted in a markedly greater extent of IBN detectable in the systemic circulation compared to all other formulations investigated. This observation might be explained considering the dissolution profiles of SNAC and IBN.

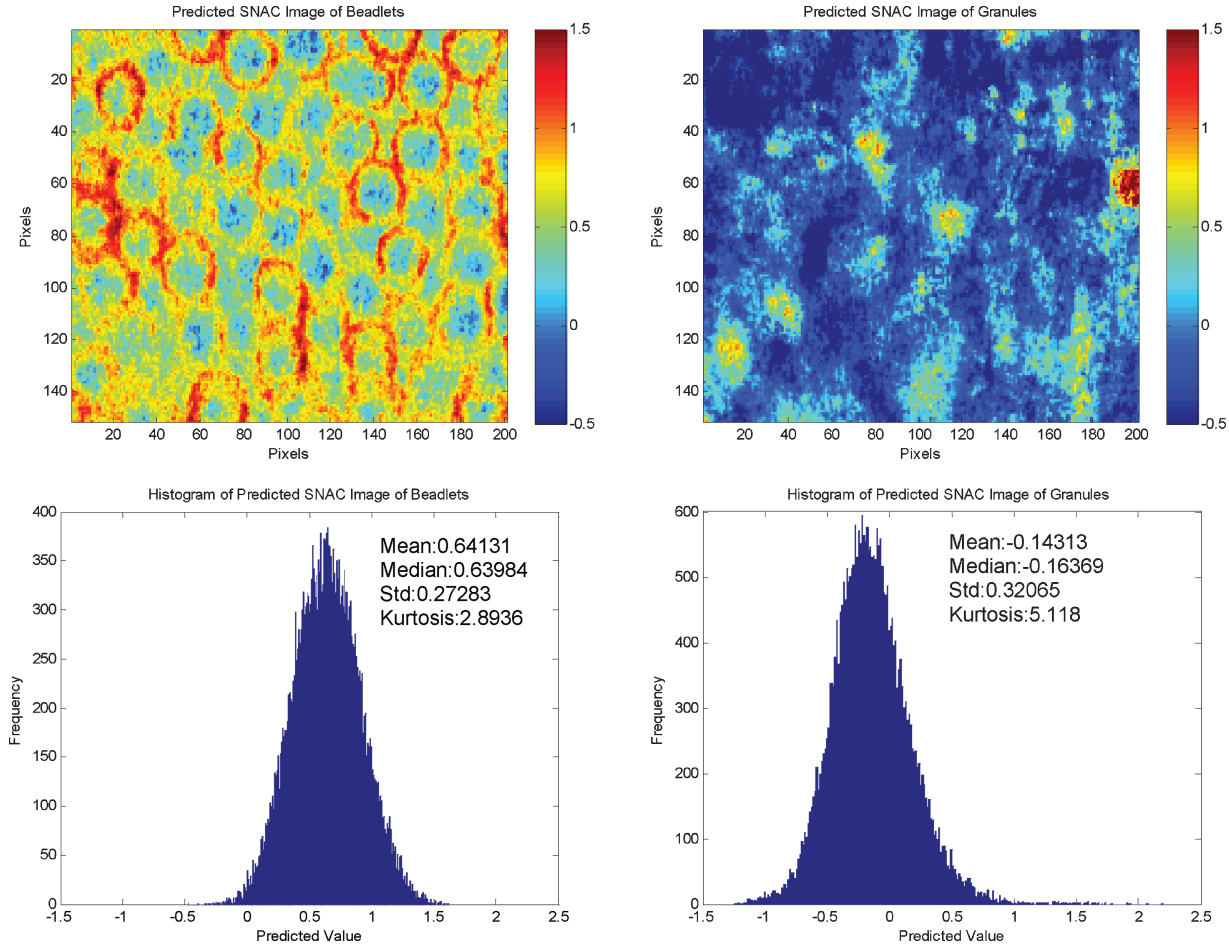

Fig. 2: Example of score images of SNAC generated from one sample image using the developed discriminant PLS model

Table 1: Summary of pharmacokinetic parameters (mean ± S.D.) of IBN in serum by treatment in cohort I

Treatment[a]	A N = 19	B N = 19	C N = 19	D N = 19
C_{max} (ng/mL)	68.7 ± 36.6	82.1 ± 35.9	56.8 ± 27.3	116 ± 88.3
Median	69.9	75.0	54.3	89.0
Range	(8.69–131)	(17.8–165)	(2.72–115)	(28.6–378)
CV (%)	53.3	43.7	48.1	76.2
T_{max} (h)	0.356 ± 0.0531	0.325 ± 0.0386	0.456 ± 0.310	0.875 ± 0.224
Median	0.333	0.333	0.333	0.983
Range	(0.333–0.500)	(0.167–0.350)	(0.167–1.50)	(0.333–1.02)
CV (%)	14.9	11.9	68.0	25.6
AUC_{last} (h*ng/mL)	96.7 ± 54.2	112 ± 44.8	86.8 ± 41.6	243 ± 125
Median	89.0	97.5	86.5	204
Range	(16.1–207)	(31.6–194)	(8.67–175)	(81.8–571)
CV (%)	56.1	40.1	47.9	51.4
$t_{1/2}$ (h)	14.6 ± 7.00	13.2 ± 4.22	13.1 ± 5.48	10.5 ± 3.70
Median	13.9	13.2	12.7	10.5
Range	(3.22–30.6)	(6.74–24.0)	(3.37–24.7)	(3.46–19.0)
CV (%)	47.9	32.0	41.8	35.3

[a] A: 30/150 mg IBN/SNAC; B: 30/300 mg IBN/SNAC; C: 30/600 mg IBN/SNAC; D: 150 mg IBN A, B and C: film-coated tablets; D: reference

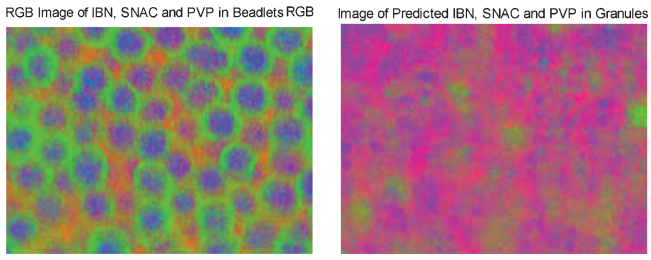

Fig. 3: Example of RGB images of IBN (red), SNAC (green), and PVP (blue), in beadlet and granule formulations

With the tablet formulations 1 and 2, IBN dissolution starts about 30 min post-dose only. Given that food was given already 15 min post-dose, the amount of IBN availble for absorption was limited. By contrast, both for the beadlets and graulate formulations, IBN dissolution started immediately upon exposure to the test fluid. While with the granulate formulation IBN is almost quantitatively released prior to the start of marked SNAC dissolution at about 30 min post-dose, with the beadlets formulation IBN and SNAC dissolve slowly and with a parallel release pattern. Thus the presence of SNAC may have protected in parts complexation of IBN once food was administered at 15 min post-dose. NIR imaging that was conducted to gain further insight into the different absoprtion behavior from the beadlets and granulate formulations showed a more uniform and intimate interaction between SNAC and IBN in the beadlet formulation. Such close contact between IBN and SNAC seems to have facilitated the formation of an IBN/SNAC complex to improve the IBN permeability *in vivo*, which potentially led to the enhanced bioavailability.

The cross-cohort food effect assessment comparing a 15, 30 and 60 min post-dose fasting duration demonstrated that at 15 min after drug administration absorption from the gastrointestinal tract is not yet complete. This is shown by the more than 2-fold higher AUC when applying a 30 and 60 min fast compared to the 15 min fast. A reduction of the 60 min post-dose fasting duration to 15 min for the novel SNAC formulation is therefore not recommended. Patients who would not comply with the fasting restrictions and eat later than 15 min after drug administration, would have markedly higher IBN plasma levels

Table 2: Summary of pharmacokinetic parameters (mean ± S.D.) of IBN in serum by treatment in cohort II

Treatment[a]	A N = 17	B N = 17	C N = 17	D N = 17
C_{max} (ng/mL)	61.6 ± 51.7	102 ± 45.0	53.2 ± 23.4	73.3 ± 46.4
Median	66.0	104	50.5	69.0
Range	(1.80–187)	(19.5–187)	(23.2–107)	(18.5–173)
CV (%)	83.9	44.1	44.0	63.3
T_{max} (hr)	0.338 ± 0.104	0.355 ± 0.0889	0.393 ± 0.0815	0.938 ± 0.684
Median	0.333	0.333	0.333	0.667
Range	(0.167–0.50)	(0.333–0.700)	(0.333–0.50)	(0.167–3.00)
CV (%)	30.8	25.1	20.7	72.9
AUC_{last} (hr*ng/mL)	74.2 ± 55.6	120 ± 43.9	76.7 ± 44.5	187 ± 106
Median	68.9	118	69.2	150
Range	(2.07–199)	(28.5–204)	(25.0–210)	(54.4–441)
CV (%)	74.9	36.7	58.1	56.8
$t_{1/2}$ (hr)	12.0 ± 6.52	13.9 ± 3.76	12.5 ± 6.97	13.5 ± 6.65
Median	12.3	14.1	10.7	12.4
Range	(1.78–27.8)	(3.83–19.8)	(3.86–30.1)	(4.17–35.3)
CV (%)	54.3	27.0	55.8	49.3

[a]. A: 30/150 mg IBN/SNAC; B: 30/300 mg IBN/SNAC; C: 30/600 mg IBN/SNAC; D: 150 mg IBN. A, B and C: film-coated tablets; D: reference

Table 3: Summary of pharmacokinetic parameters (mean ± S.D.) of IBN in serum by treatment in cohort III

Treatment[a]	A N = 19	B N = 19	C N = 19	D N = 19
C_{max} (ng/mL)	259 ± 127	197 ± 142	147 ± 59.4	116 ± 149
Median	277	158	146	76.4
Range	(42.5–500)	(12.2–571)	(52.2–263)	(27.9–627)
CV (%)	49.0	72.1	40.3	128
T_{max} (hr)	0.308 ± 0.0919	0.360 ± 0.121	0.363 ± 0.112	0.661 ± 0.264
Median	0.25	0.333	0.333	0.667
Range	(0.25–0.583)	(0.250–0.667)	(0.250–0.700)	(0.250–0.983)
CV (%)	29.8	33.7	30.9	39.9
AUC_{last} (hr*ng/mL)	287 ± 150	227 ± 149	178 ± 59.0	223 ± 203
Median	236	192	182	152
Range	(40.0–681)	(23.3–486)	(82.8–332)	(60.6–825)
CV (%)	52.4	65.5	33.2	91.2
$t_{1/2}$ (hr)	9.12 ± 2.26	10.7 ± 5.36	9.68 ± 3.06	7.77 ± 3.31
Median	9.31	9.00	10.2	8.58
Range	(3.51–12.2)	(4.82–26.1)	(3.51–14.9)	(3.50–13.8)
CV (%)	24.8	50.1	31.6	42.6

[a)] A: 30/150 mg IBN/SNAC; B: 30/300 mg IBN/SNAC; C: 30/600 mg IBN/SNAC; D: 150 mg IBN. A, B and C: Beadlets in capsules; D: reference

than those adhering to the dosing instructions. The finding that the IBN plasma concentration-time profiles are superimposable when applying a 30 or 60 min post-dose fast demonstrates that absorption is complete at 30 min post-dose, and there is no need to wait more than 30 min prior to food intake with the new IBN/SNAC formulation.

In summary, this study has shown that when combined with the carrier molecule SNAC, the systemic exposure to IBN could be markedly increased. With the SNAC formulation, an oral IBN dose of 30 mg resulted in a more than 2-fold greater exposure compared to the approved 150 mg tablet formulation. A prerequisite for the improved absorption from the gastro-intestinal tract is the use of an optimal formulation type, namely drug-coated beadlets presenting the homogenous IBN/SNAC mixture at their surface, this way allowing a fast release and subsequent absorption already in the upper part of the gastro-intestinal tract. The exact IBN dose that results in comparable AUC to the approved tablet formulation will be determined in a subsequent trial in healthy post-menopausal females.

4. Experimental

4.1. Subjects

Ninty-seven healthy male and female subjects were enrolled in this study. Subjects were in good general health as assessed by a physician on the basis of a medical history, general physical examination, vital signs, ECG and laboratory tests. Subjects were excluded in case of a history of clinically significant gastro-intestinal, cardiovascular, musculoskeletal, endocrine, hematological, psychiatric, hepatic, broncho-pulmonary, neurological, renal or allergic disease including aspirin-sensitive asthma, abnormalities of the oesophagus which could delay oesophageal emptying such as stricture or achalasia, active upper gastrointestinal disease such as dysphasia, gastritis, duodenitis or ulcers. Subjects who participated in a clinical study with an investigational drug within 6 weeks or 6 times the elimination half-life (whichever was longer) of first drug administration or who donated/lost more than 400 mL of blood in the 3 month period prior to first drug administration were not allowed to enter the trial. Each subject gave written informed consent. The protocol was approved by the Upper South B Regional Ethics Committee, Ministry of Health, Christchurch, New Zealand. The study was conducted in accordance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the Declaration of Helsinki.

Table 4: Summary of pharmacokinetic parameters (mean ± S.D.) of IBN in serum by treatment in cohort IV

Treatment[a]	A N = 19	B N = 19	C N = 19	D N = 19
C_{max} (ng/mL)	364 ± 123	340 ± 123	86.7 ± 59.5	20.9 ± 15.8
Median	366	349	75.4	17.0
Range	(157–571)	(136–619)	(13.2–226)	(8.14–76.0)
CV (%)	33.8	36.1	68.6	75.4
T_{max} (hr)	0.356 ± 0.134	0.345 ± 0.128	0.446 ± 0.224	0.611 ± 0.0971
Median	0.333	0.333	0.333	0.667
Range	(0.167–0.667)	(0.167–0.667)	(0.250–1.02)	(0.333–0.683)
CV (%)	37.6	37.0	50.1	15.9
AUC_{last} (hr*ng/mL)	561 ± 245	518 ± 293	123 ± 66.0	40.4 ± 26.2
Median	542	447	129	31.6
Range	(152–1140)	(157–1240)	(26.5–271)	(19.1–132)
CV (%)	43.7	56.7	53.5	64.8
$t_{1/2}$ (hr)	8.98 ± 1.78	9.20 ± 1.33	9.41 ± 3.02	15.5 ± 7.82
Median	8.87	9.46	9.03	13.0
Range	(6.37–14.0)	(7.37–11.6)	(3.36–15.4)	(7.44–36.6)
CV (%)	19.9	14.4	32.1	50.4

[a)] A: 30/150 mg IBN/SNAC, cohort III formulation (drug-coated beadlets), 60 min fast; B: 30/150 mg IBN/SNAC cohort III formulation, 30 min fast; C: 30/150 mg IBN/SNAC, granulate formulation; D: 50 mg IBN

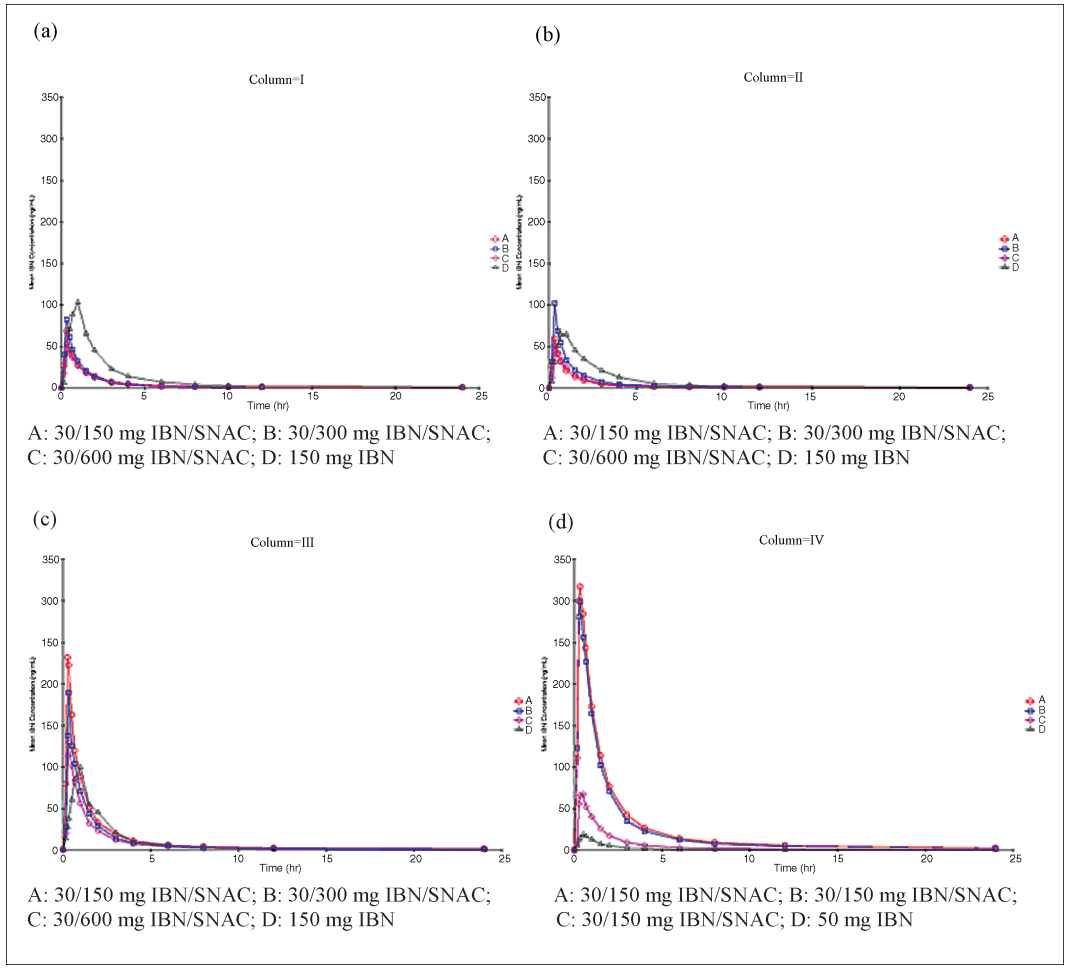

Fig. 4: Mean serum concentration-time profiles of IBN by treatment in (a) cohort I, (b) cohort II, (c) cohort III, (d) cohort IV

4.2. Study design

This was a single centre, open-label, randomized, four-cohort study. Within each cohort, formulations were investigated in a four-period crossover design. Subjects underwent a thorough screening medical examination between day –28 and day –2 of the study. Three different types of oral formulations (1 [film coated tablet], 2 [film coated tablet], and 3 [drug-coated beadlets in a hard gelatin capsule]) with 30 mg of ibandronate were studied in cohorts I to III. For each type of formulation, three different ratios of IBN:SNAC (1:5 [30/150 mg], 1:10 [30/300 mg] and 1:20 [30/600 mg]) were tested. The reference formulation in cohorts I to III was the 150 mg IBN tablet approved for the osteoporosis indication (Table 6).

Based on the results from cohorts I to III, the cohort III formulation (drug-coated beadlets in a hard gelatin capsule) was further evaluated, in cohort IV, to assess the impact of the timing of food intake on the absorption of IBN. The protocol was amended to include a modified hard gelatin capsule formulation (granulate) with 30 mg ibandronate at a ratio of IBN:SNAC of 1:5 (i.e., 30/150 mg, formulation 4) into cohort IV. To also generate data that allow a direct comparison to the 50 mg IBN tablet approved for the oncology indication, this tablet was used as the reference treatment D in cohort IV.

In cohorts I, II and III subjects received a standardized breakfast 15 min after drug intake for the SNAC containing formulations in treatment arms A, B and C. For the reference treatment arm D, a standardized breakfast was given 60 min after drug intake as per clinical practice. For the food effect assessment in cohort IV, a standardized breakfast was given 30 min (treatments B and D) or 60 min (treatments A and C) after drug intake. Subjects received 500 mg supplemental calcium once-daily throughout the course of the study. Table 6 details the treatments and dosing conditions in the different cohorts.

4.3. Blood sampling and determination of IBN concentrations

On Day -1, a 45 mL venous blood sample was collected to create calibration curves for the enzyme-linked immunoabsorbent assay (Ibandronate ELISA, Microcoat GmbH, Bernried, Germany) for each individual. On Day 1 of each treatment period, 5 mL venous blood samples were collected in 5 mL plain glass Vacutainer tubes at pre-dose and at 10, 15, 20, 30, 40, 60 min, 1.5, 2, 3, 4, 6, 8, 12 and 24 h post-dose. Serum obtained from these blood samples were assayed for levels of IBN. The calibration range was 25–1600 pg/mL (including "0" standard). The lower limit of quantification for this assay was 50 pg/mL. Quality control serum samples at three different concentrations (150, 300 and 600 pg/mL) were analyzed with each assay batch (approx. 8% quality controls referenced to the number of unknown samples). Results from study samples were only accepted if not more than 33% of the quality control samples within each assay batch had an inaccuracy greater than 15%.

4.4. Pharmacokinetics

Pharmacokinetic parameters were estimated using non-compartmental methods according to the Roche internal "Guideline for calculation and analyses of non compartmental pharmacokinetic parameters" issued April 2005 and using WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA).

Primary pharmacokinetic parameters for IBN were Cmax (maximum plasma concentration) and AUClast (area under the plasma concentration-time curve from time zero to the time of the last measurable plasma concentration). Secondary pharmacokinetic parameters were tmax (time of maximum plasma concentration) and t1/2 (elimination half-life).

4.5. Statistical methods

The ANOVA model:

$$y_{ijklm} = \mu' + \alpha_i + s_{(j)} + \tau_k + \pi_l + \varepsilon_{ijklm}$$

$$(\mathrm{i} = \mathrm{f, m;\ j} = 1, .., \mathrm{N;\ k} = \mathrm{A,\ B,\ C,\ D;\ l} = 1, 2, 3, 4)$$

was applied to the logarithmically transformed variables AUC_{last} and C_{max}, where μ' denotes the general mean of the transformed variable, α_i the gender effect, s_j the effect of subject j (nested in gender), τ_k the direct effect of treatment k, π_1 the effect of period l, ε_{ijklm} the random deviation and N the number of subjects included into the analysis.

Table 5: Estimated ratios for AUClast and Cmax with respect to treatment by cohort

Cohort	Treatment Ratio	AUClast Estimate	AUClast 90% confidence interval	
			Lower limit	Upper limit
I	A/D	0.260	0.177	0.381
	B/D	0.455	0.308	0.673
	C/D	0.342	0.231	0.505
II	A/D	0.212	0.093	0.485
	B/D	0.646	0.415	1.007
	C/D	0.528	0.344	0.808
III	A/D	1.372	0.993	1.896
	B/D	0.889	0.580	1.363
	C/D	0.898	0.690	1.168
IV	A/D	14.232	10.833	18.698
	B/D	12.614	9.601	16.572
	C/D	2.915	2.219	3.829
Cohort	Treatment Ratio	Cmax Estimate	Cmax 90% confidence interval	
			Lower limit	Upper limit
I	A/D	0.386	0.247	0.603
	B/D	0.872	0.594	1.280
	C/D	0.550	0.318	0.950
II	A/D	0.340	0.131	0.881
	B/D	1.409	0.829	2.394
	C/D	1.056	0.669	1.666
III	A/D	2.629	1.830	3.776
	B/D	1.571	0.959	2.573
	C/D	1.506	1.080	2.101
IV	A/D	19.467	15.076	25.138
	B/D	18.176	14.155	23.338
	C/D	3.788	2.612	5.494

The *estimated population relative bioavailability* of the test treatment k was defined as $\mu_k/\mu_D = \exp(\tau_k - \tau_D)$, where μ_k (k = A, B, C, D) denotes the population (geometric) means of treatment k for the untransformed variables.

The primary parameters for assessing the relative bioavailability were the area under the concentration-time curve AUC_{last} and C_{max}. The relative bioavailability was defined as the ratio $\frac{AUC_k}{AUC_D}$ where the subscript k stands for a test formulation and D for the reference formulation (Bonviva® 150 mg in cohorts I to III and Bondronat® 50 mg in cohort IV). The ratios $\frac{Cmax_k}{Cmax_D}$ were treated in the same way.

Other pharmacokinetic parameters were regarded as secondary.

Table 6: Doses and dosing conditions in cohorts I to IV

Cohort	Formulation	Treatment	Fasting duration after drug intake
Cohort 1	Film coated tablet (1)	A: 30 mg IBN:150 mg SNAC	15 min
	Film coated tablet (1)	B: 30 mg IBN:300 mg SNAC	15 min
	Film coated tablet (1)	C: 30 mg IBN:600 mg SNAC	15 min
	Reference	D: 150 mg IBN	60 min
Cohort II	Film coated tablet (2)	A: 30 mg IBN:150 mg SNAC	15 min
	Film coated tablet (2)	B: 30 mg IBN:300 mg SNAC	15 min
	Film coated tablet (2)	C: 30 mg IBN:600 mg SNAC	15 min
	Reference	D: 150 mg IBN	60 min
Cohort III	Beadlets in capsules (3)	A: 30 mg IBN:150 mg SNAC	15 min
	Beadlets in capsules (3)	B: 30 mg IBN:300 mg SNAC	15 min
	Beadlets in capsules (3)	C: 30 mg IBN:600 mg SNAC	15 min
	Reference	D: 150 mg IBN	60 min
Cohort IV	Beadlets in capsules (3)	A: 30 mg IBN:150 mg SNAC	60 min
	Beadlets in capsules (3)	B: 30 mg IBN:150 mg SNAC	30 min
	Granulate in capsules (4)	C: 30 mg IBN:150 mg SNAC	60 min
	Reference	D: 50 mg IBN	30 min

Table 7: IBN/SNAC formulations

Formulation	Technology	Excipients
1: Film-coated tablets (cohort I)	Aqueous spray granulation in fluid bed dryer; Water sprayed onto powder bed of intragranular materials (including IBN and SNAC)	SNAC, lactose monohydrate, croscarmellose sodium, povidone, sodium stearyl fumarate, hypromellose, macrogol, titanium dioxide
2: Film-coated tablets (cohort II)	Aqueous spray granulation in fluid bed dryer; IBN dissolved in water prior to spray granulating the remaining intragranular materials	SNAC, lactose monohydrate, croscarmellose sodium, povidone, sodium stearyl fumarate, hypromellose, macrogol, titanium dioxide
3: Drug-coated beadlets in hard gelatin capsules (cohorts III and IV)	Spray coating of IBN and SNAC solution in water onto the beadlets in fluid bed dryer	SNAC, microcrystalline cellulose (as beadlets), povidone, talc, calcium silicate; gelatin, titanium dioxide
4: Granules in hard gelatin capsules (cohort IV)	Aqueous spray granulation in fluid bed dryer; Water sprayed onto powder bed of intragranular materials (including IBN and SNAC)	SNAC, croscarmellose sodium, povidone, sodium stearyl fumarate, calcium silicate

4.6. Formulation

IBN was supplied for this study as either oblong, white to off-white film-coated tablets or white hard gelatin capsules. Labeled strength was based on the free acid (e.g., a 30 mg film-coated tablet contains 30 mg ibandronic acid in the form of 33.76 mg ibandronate monosodium salt monohydrate). In addition to the drug substance, each tablet did contain SNAC (sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate) as an excipient (IBN:SNAC ratios of 1:5, 1:10, and 1:20). All formulation principles are summarized in Table 7.

4.7. Dissolution testing

Dissolution testing was performed on each formulation using the flow-through cell apparatus (USP Dissolution Apparatus IV) in media and flow conditions simulating fasted state conditions. In a sequential manner, the media, which was pumped through the cell at 6 mL/min, was changed over from simulated gastric fluid (SGF) to maleate buffer pH 6.5 after 20 min to simulate the transition of the dosage form from the stomach to the small intestine. Collected samples were analyzed for dissolved IBN by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and for SNAC by high-performance liquid chromatography (HPLC).

4.8. Chemical imaging characterization of the formulation

Near infrared (NIR) chemical imaging was employed to examine distribution of IBN and SNAC in the different formulations. NIR chemical images were collected for each sample using the Sapphire^{TM} NIR chemical imaging system (Malvern Instruments Inc., Columbia, MD) at a wavelength range of 1850 to 2200 nm. The image spatial resolution was 20 μm/pixel with the field of view of 5.1 × 6.4 mm. To eliminate the spectra variation due to physical interferences and to enhance spectra characteristics, all sample spectra were pre-processed using standard normal variate and Savitsky-Golay first derivative (7,2,1).

A discriminant partial least square (PLS) model was developed using pure component spectra of IBN, SNAC, and another main excipient, polyvinylpyrrolidine (PVP). The statistical parameters of each SNAC PLS score image were calculated as a measure of the degree of SNAC distribution in the formulation. Red-green-blue (RGB) images simultaneously mapping IBN, SNAC and PVP, respectively, were generated to get additional information on the distribution of the three components in the capsule formulations tested in cohorts III (drug-coated beadlets) and IV (granulate). Principle component analysis (PCA) was performed on the sample spectra to determine whether the physical and chemical properties of beadlet and granule formulations are intrinsically distinctive.

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