Response to Letter to the Editor: ‘About oral absorption and human pharmacokinetics of chondroitin sulfate’

Dr Volpi’s comments will be considered one by one as a Response under a restatement of each comment in italics:

1. “However, many previous studies on man [see Refs. 2 and 3], animals4 and in vitro models5 using CS or similar natural biomacromolecules, i.e., dermatan sulfate, desulfated CS, fucosyl CS, heparin, or mixtures [see Refs. 2 and 3] clearly demonstrated the oral absorption of these polysaccharides. As a consequence, how is it possible to explain the “absence” of CS oral absorption in man observed in1?”

Response
The previous studies on man (Refs. 2 and 3) are the only studies considered relevant to the clinical study presented in this paper. Studies on animals⁴ and in vitro⁵ or with non-CS polysaccharides, while informative, are not directly relevant.

In both Refs. 2 and 3 the studies involved 20 healthy volunteers given a single high dose (4000 mg) and plasma levels were determined over the first 48 h.

This dosage is 10-fold higher than the single clinically-relevant dose (400 mg) administered in GAIT. GAIT patients took oral doses of 400 mg three times per day for a total of 1200 mg over approximately 15 h.

It is for these dosage reasons that we did not refer to Dr Volpis studies in our discussion, although we did acknowledge (our Ref. 14) Dr Volpis development of an HPLC/MS method for analysis of CS and HA disaccharides using 2-aminoacridine tagging, the reagent used with FACE analysis in our paper.

2. “Under these conditions and considering that Jackson et al. determined a mean CS endogenous concentration of approx. 20 μg/ml with a possible individual variation of 5 μg/ml (25%) and 40–45% variation in AUC values after CS administration (see Tables IV and VI)1 (similar values to those obtained in studies2,3), it is hard to obtain any significant plasma CS variation. To confirm this, a trend towards higher CS concentrations was observed by the authors but differences were found to be non-significant, also perhaps due to the small number of subjects, 9–10, studied11.”

Response
Dr Volpi has calculated (with inherent assumptions about the pharmacokinetics of oral CS taken by GAIT patients) that when compared to Dr Volpis own work with 4000 mg dosing, it is not unexpected that there was no detectable change (above endogenous levels) in plasma concentrations at the 400 mg × 3 dosing regimen used in GAIT. This would appear to be confirmed by our studies. We were confident that we made this clear to readers in the early paragraphs of the Discussion. We discussed the total endogenous concentration of CS (10–20 μg/ml), and (if the pharmacokinetics for CS and GlcN are similar) the expected peak of absorbed CS (200 ng/ml), after oral dosing at 400 mg. It should be noted however that we provide other data (regarding the disaccharide composition) which also suggest little or no absorption of oral CS when taken at clinically-relevant doses.

3. “Along with detection limits, other factors may influence CS recovery from plasma. For example, previous studies, contrary to research in Ref. 1, accurately avoid the use of heparin (a polysaccharide with similar anionic properties to CS), interfering in particular with the extraction procedures”.

Response
We are unaware of such previous studies and since no references are provided by Dr Volpi we cannot answer this potential criticism of our work.

4. “Furthermore, Jackson et al. measured a CS endogenous amount of ~20 μg/ml, virtually 2–4 times more than several other studies performed by various analytical approaches [see Refs. 2 and 3].”

Response
We consider that the difference in mean concentration of endogenous CS found in our studies (20 ±5 μg/ml) to those in Osteoarthritis and Cartilage 2002; 10: 768–777 (0.3–5.3 μg/ml), and those in Osteoarthritis Cartilage 2003; 11: 433–441 (1.53–3.37 μg/ml) to be reasonable given the following considerations. Firstly, the human populations under study are from different age groups, different ethnic groups and in different countries with different dietary patterns. In addition the Methods of CS isolation from the plasma (acetone precipitation vs Superose 6 chromatography) and the methods of quantitative analysis (FACE vs HPLC) were different in the different studies. Given that these are different multi-step biochemical methods, a difference between the concentrations determined in the two laboratories of 4-fold is not unexpected or cause for concern.

5. “Additionally, due to the anionic properties of these macromolecules, absorbed CS may interact with several blood components, in particular with endothelium reported, for example, to have the power to remove up to 80% heparin from circulation after administration”.

Response

the plasma compartment may be rapidly taken up by liver cells where polysaccharides are most recovered in experimental animals.

Response

We are in agreement with Dr Volpi that there are many potential cellular activities in the gut, portal vein and post-hepatic circulation which could markedly limit the circulating concentration of the oral dose Cs. We make reference to this point in the conclusions section of the opening Summary to the paper.

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