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Influence of ageing on the gastrointestinal environment of the rat and its implications for drug delivery

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ABSTRACT

Age-mediated changes in gut physiology are considerations central to the elucidation of drug performance from oral formulations. Using rats of different age groups we measured the pH, buffer capacity, fluid volume, osmolality, and surface tension of gastrointestinal (GI) fluids, and therein explored the impact of these variables on prednisolone and mesalazine solubility in luminal fluids. We also studied the distribution of gut associated lymphoid tissue (GALT) and mucus layer thickness across the GI tract in rats of different age groups. At a mucosal level, there was an increase in GALT from young to adult rat. Gastrointestinal pH and buffer capacity remained mostly unchanged with age, except some pH differences in stomach and distal small intestine and a higher buffer capacity in the large intestinal fluids of young rats. Osmolality and surface tension also remained unaffected with the exception of a lower osmolality in elderly stomach and a lower surface tension in the small intestine of young rats. The difference in luminal environment on ageing influenced the solubility of studied drugs, for instance prednisolone solubility was shown to be higher in adult rats (mid small intestine and caecum) and solubility of mesalazine was significantly higher in the elderly distal small intestine.

1. Introduction

As we age, the gastrointestinal tract undergoes various morphological and functional changes (Majumdar and Basson, 2006; Newton, 2004), which may disturb normal homeostatic mechanisms, and so predispose the gut to the development of certain diseases (Pilotto, 2004; Staff and Shaker, 2001). A general decline in gastrointestinal function also parallels the ageing process, involving circumstances such as delayed gastric emptying, reduced splanchnic blood flow, changes in GI motility besides other bodily changes, such as serum albumin, hepatic and renal function, body mass and fat, which significantly impact upon the pharmacokinetics of certain drugs (Gidal, 2006; Roy and Varsha, 2005; Smits and Lefebvre, 1996; Wilkinson, 1997). It is also reported that intestinal permeability changes significantly with age in rats (Annaert et al., 2010), though such an effect has yet to be observed in humans. We know that the intra-luminal environment influences the drug bioavailability and the performance of orally-administered formulations (McConnell et al., 2008c; Varum et al., 2013). Therefore an understanding of the gastrointestinal environment for members of different age groups is vital for the successful delivery of drugs to achieve required therapeutic outcomes in different patient categories – broadly, the young, adult and elderly.

On the one hand, it is not always possible to experiment in humans, to which end gastrointestinal fluids are often instead obtained from human volunteers to enable study of luminal characteristics. However, though such fluids are considered very useful, it is generally difficult to acquire them from healthy subjects, and from the length of the entire gastrointestinal tract. Animal models, therefore, provide an invaluable compensatory resource as 'surrogates' for human subjects, and among the different species typically studied, the rat is one of the most extensively used animal models in biomedical research. It is also widely regarded as easier to perform age-mediated studies in rats due to their relatively shorter life span (Festing et al., 2002). Furthermore, it has also been reported that drug absorption in rats closely resembles to that in human for a variety of compounds. Chiou and Barve (1998) found a good correlation between rat and human absorption for 64 test compounds, and those with complete absorption ($n = 40$) showed much stronger correlation than those ($n = 24$) with incomplete absorption (<90%) ($r^2 = 0.97$ and 0.89, respectively). The linear

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relationship between human and rat absorption was also identified by Zhao et al. (2003), where the absorption of 241 compounds in the human deviated from absorption in rats by only 11%.

By using rats as models, we have within the context of this paper explored the effect of age on the gastrointestinal luminal environment, involving the study of pH, fluid volumes, osmolality, surface tension and buffer capacity of the gastrointestinal fluids in young, adult and elderly rats. We have also measured the solubility of two model drugs – prednisolone (neutral) and mesalamine (ionisable) – in luminal fluids from rats of different age groups and therein explored the impact of these variables on drug solubility in luminal fluids. On the mucosal level, the distribution of gut-associated lymphoid tissues (GALT) was determined, and mucus layer thickness was also measured across the age groups. Where mucus layer serves as a physical barrier to permeation and if obeying Fick's first law, its varying thickness may affect drug absorption. GALT on the other hand servers not only as a first line of defense against infiltrating pathogens and has applications in oral vaccination (McConnell et al., 2008a,b; Merchant et al., 2011) but is also exploited to improve the bioavailability of lipophilic compounds by avoiding first-pass metabolism (Ali Khan et al., 2013; Faisal et al., 2010).

2. Materials and methods

2.1. Materials

Mesalazine (mesalamine, 5-ASA) was obtained from Sigma–Aldrich Co., Ltd. (Dorset, UK) and prednisolone was obtained from Aventis Pharma (Antony, France). Acetic acid (BDH Porlabo, Briare, France), calibration fluids for pH 4.01 and 7.01 (VWR, Dorset, UK), O.C.T. compound (cryogel), glacial acetic acid and poly-L-lysine slides were purchased from VWR International. Sodium metabisulfite and periodic acid were obtained from Sigma–Aldrich, UK. Clearmount mounting solution was purchased from Invitrogen, Paisley, UK. Schiff's reagent, Alcian Blue 8GX and paraformaldehyde were obtained from Fisher Scientific, UK.

2.2. Animals

Healthy, male Wistar rats of different age groups – young, 4 weeks; adult, 8 weeks; and elderly, 38 weeks – were procured from Harlan UK Ltd. (Oxfordshire, UK) and maintained on a rodent diet (Teklad Global, 18% Protein) from Harlan Ltd. (Oxfordshire, UK). The average longevity of a laboratory rat is between 2 and 3 years (Festing et al., 2002), though an age of 38 weeks was chosen for the elderly group studied here due to a commonly associated decrease in survival rates of animals after 52 weeks of age. A general decline in physiological function parallels the ageing process and is a consequence of general frailty in elderly population (Majumdar and Basson, 2006; Newton, 2004; Pilotto, 2004; Staff and Shaker, 2001). The 38 weeks old elderly group, therefore, represented healthy and active old-aged subjects instead of frail geriatrics. Annaert et al. (2010) have also used 38 weeks old rats to represent elderly subjects in a study to explore the age related differences in intestinal permeability.

All procedures were conducted in accordance with the Home Office standards under the Animals (Scientific Procedures) Act 1986. Studies were performed in 14 rats each from the 4 weeks, 8 weeks and 38 weeks categories. The animals were restrained in the laboratory for 1 week before the commencement of any experiment in order to allow the animal to adjust to a new environment, and therefore to minimise the influence of changes in feeding behaviour. Food and water was available *ad libitum* to every animal involved in the studies.

2.3. Animal handling, dissection and sample preparation

On the days of study the animals were killed and the gastrointestinal tracts immediately sectioned into the stomach, small intestine, caecum, and colon, with the small intestine and colon being further subdivided. The pH was measured *in situ* during dissection. The dimensions of the different regions of the gastrointestinal tract were measured *post-mortem* and grouped to analyse separately for young, adult and elderly rats. This is to be noted that *post-mortem* measurements tend to be different from those in living subjects due to relaxation of smooth muscles. However, the measurements were performed immediately after euthanasia to minimise such effects.

The length and width (at maximum point) of the stomach was measured using a measuring tape and the length of the lesser and greater curvatures were measured using a string. The distances measured in both cases spanned from the cardia to the pylorus along either curve, and when measuring the length of the greater curvature, care was taken to pull down the anterior face of the stomach. The length of the string was then measured to find out the exact length of the curvatures, and the length and the width (at maximum point) of the caecum were measured using measurement tape. The emptied gastrointestinal sections were rinsed, opened length ways and the length and width (circumference) subsequently measured using measurement tape.

Gastrointestinal fluids obtained from six rats of the young, adult and elderly groups were dried in order to measure fluid volume distribution in the gut, as per the procedure detailed in the following section. Fluids from the remaining rats ($n = 8$ each group) were pooled according to anatomical locations, including the stomach, small intestine (proximal, mid and distal), caecum, and colon, and utilised for further characterisation. The buffer capacity, osmolality, and surface tension were also measured according to same procedures as outlined below. The solubility of two drugs, prednisolone and mesalazine, were also measured in these fluids.

2.4. pH of the gastrointestinal luminal contents

The pH was measured *in situ* by placing the pH probe (FC202, designed for viscous and semi-solid materials, Hannah Instruments, Bedfordshire, UK) within the luminal contents of each gastrointestinal section using a calibrated pH meter (HI HI99161, Hanna Instruments, Bedfordshire, UK). The electrode was washed with distilled water and recalibrated between measurements.

2.5. Gastrointestinal water and solid contents

Following pH measurement *in situ*, the contents from the different gastrointestinal sections were separately emptied into pre-weighed containers, and their corresponding wet mass recorded. Subsequently, the gastrointestinal contents were lyophilised (Virtis-Advantage-EL Lyophilizer, Virtis, UK) and the dry mass recorded. The water content was then calculated as the difference between the wet and dry masses for each gastrointestinal segment.

2.6. Osmolality

Osmolality of the supernatants from the gastrointestinal fluids was determined with use of a Digital Micro-Osmometer (Type 5R) (Hermann Roebling MESSTECHNIK, Berlin, Germany), where osmolality is measured exploiting the theory of freezing point depression. Samples were thawed to room temperature before measurements were conducted, and subsequently centrifuged (at 4472 rcf/5000 rpm for 10 min) to obtain the supernatants. A volume of 100 μL was used for each measurement.