Title: Physiological adaptations affecting drug pharmacokinetics in space: what do we really know? A critical review of the literature.

Running title: Drug PK in space medicine

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Abstract

As human spaceflight continues with extended mission durations, the demand of effective and safe drugs is going to increase. To date, the medications used during missions (for space motion sickness, sleep disturbances, allergies, pain and sinus congestion) are administered under the assumption that they act similarly as on the Earth. During spaceflights however fluid shifts, muscle and bone loss, immune system dysregulation and changes in the gastrointestinal tract and metabolism are documented. These alterations may change the pharmacokinetics (PK) and pharmacodynamics. The information gained from bed-rest studies and from inflight observations is partial and demonstrates variability in drug PK. The objectives of this review are to report: i) the impact of the space environmental stressors on human physiology in relation to PK; ii) the state-of-the-art on experimental data in space and/or in ground-based models; *iii*) the validation of ground-based models for PK studies; and *iv*) the identification of possible research gaps.

Keywords: pharmacokinetics, space stressor, drugs, formulations, animal studies, bed rest studies, real evidence in space

1. Introduction

When exposed to the space environment, all systems and organs undergo adaptations that can affect drug pharmacokinetics (PK) and thus their efficacy (Putcha & Cintrón, 1991; Derendorf, 1994; Czarnik & Vernikos, 1999; Graebe, Schuck et al., 2004; Kast, Yu et al., 2017; Strollo, Gentile et al., 2018). Beside weightlessness, other space environmental factors and stressors can influence the response to drugs. The highly demanding and stressful work, isolation and confinement, forced interpersonal relationships, lack of circadian cycle and exposure to artificial light and noises heavily affect astronaut's mood and central nervous system well-being, with consequences on all the organs and physiological systems. Reduced sanitation and exposure to radiation alter human microbial balance, increase the risk of DNA damage, thus compromising immune system function (Williams, Kuipers et al., 2009). Relative mild chronic hypoxia can be a further stressor. It is known that the physiologic changes caused by high altitude hypoxia on the Earth are quite similar to the ones found in space. Alterations in the absorption, distribution, metabolism, and excretion of drugs, require adjustment in dosage regimens to prevent toxicity or maintain efficacy in the high altitude hypoxia environment (Bailey, Stacey et al., 2018; Zhou, Nian, et al., 2018).

When given orally, by intravenous or intramuscular injection, intranasally or rectally, or as a patch, a drug must be absorbed, distribute through the body, act on receptors or other targets, be metabolized and excreted. When circulating in blood, it can variably bind to plasma proteins or red blood cells. All the above steps are influenced by the chemical features of the drug, the formulation, its ability to cross membranes and barriers, and time of contact with them. However, these last features can be significantly altered by microgravity and other space environmental stressors leading to potential modifications of drug efficacy and safety in space (Wotring, 2011; Wotring, 2018; Eyal & Derendorf, 2019; Eyal, 2020). In particular, space radiations can affect drug stability, which is an additional factor that can variably contribute to modifications of drug effects in space and whose magnitude is difficult to predict (Blue, Chancellor et al., 2019a).

The first inflight evidence that drugs may have different efficacy in space in comparison to Earth was reported by Pool and Nicogossian in 1983, analysing the biomedical results collected during the Space Shuttle Orbital Flight Test (SSOFT) program. This program included four orbital Space Shuttle flights, carried out between April 1981 and June 1982, lasting from 2 up to 7-8 days (Kennedy Space Center Historical Report No. 1B, KHR-1B, NASA). The main medical condition affecting astronauts during these short flights was space motion sickness (SMS), a type of motion sickness induced by lack of normal gravitational forces. This syndrome is characterized by increased sensitivity to motion and head movements, headache, malaise, lethargy, stomach awareness, loss of appetite, nausea, and episodic vomiting. It usually occurred in the first 6 hours of spaceflight and, if untreated, symptoms peaked in 24-48 hours and resolved in 72-96 hours. Development of SMS could potentially impair the abilities of crewmembers and negatively impact the effectiveness and safety of operations during spaceflight. Thus, each crewmember involved in the SSOFT program was tested at least once, approximately 3-6 months before the actual flight, for susceptibility to laboratory induced motion sickness. This was evaluated by the standard Coriolis Sickness Susceptibility Index (CSSI) test, consisting in the performance of head movements in four spatial planes while rotating at a constant velocity in a servo-controlled chair. The test was terminated when the subject reached malaise level 3, *i.e.* eight symptom points, of motion sickness or performed 150 head movements. Crewmembers involved in the SSOFT program displayed average CSSI of 41.4, on a 1-100 scale with

higher scores indicating subjects resistant to sickness. This score, compared to healthy non-astronaut volunteers with mean CSSI of 12.2, shows that astronauts are more resistant to motion sickness than the general population (Pool & Nicogossian, 1983). Nevertheless, four out of eight crewmembers that flew during the SSOFT program, that is 50% of exposed subjects, reported one or more SMS symptoms during flight. Six of them were pre-medicated for this condition, 5 with an oral formulation of 0.4 mg scopolamine in combination with 5 mg dextroamphetamine (scop/dex) and 1 with a transdermal scopolamine patch. Prophylaxis was considered somewhat effective in relieving SMS symptoms. However, one crewmember observed that oral scop/dex required longer time (1-1.5 hours) to be effective inflight in comparison to what experienced during ground-based testing that gave substantial beneficial effects in 30-40 min. This finding suggests that drug efficacy may be altered in space or that ground-based experimental models are not sufficiently validated as surrogate models of microgravity to predict drug efficacy in space (Blue, Bayuse et al., 2019b).

Reports are available in the literature further underlining the complexity of space medicine and how clinical decisions and pharmacological treatments are often based on known mechanism of action and PK characteristics of drugs on the Earth, without sufficient evidence on their efficacy in space. An example was reported in the case of a crewmember, without a known history of terrestrial allergies who developed a chronic rash associated with upper respiratory rhinitis and watery eyes (Crucian, Johnston et al., 2016). The rash persisted for the entire duration of the space mission, with worsening of symptoms during the most stressful operations performed on board the International Space Station (ISS), including the docking of Space Shuttle, Soyuz and cargo vehicles, five extra vehicular activities (EVAs), and operations in support of EVA's carried out by other crewmates. Hydrocortisone cream was used as initial treatment, followed by the prescription of fluconazole at day 22 without additional benefits. The rash presented with highest severity at mission day 73, that was two days after one crewmate was informed of the death of a family member. This event was considered a psychological stressor for the entire crew. Topical treatment was switched to triamcinolone acetonide cream and systemic steroids were used to control the symptoms. Alterations in the immune system were described in this astronaut, including reduced T-cell functions, altered leukocyte distribution and cytokine secretions (Crucian, Johnston et al., 2016). More recently, obstructive internal jugular venous thrombosis was diagnosed in an asymptomatic astronaut after approximately 2 months on the ISS (Auñón-Chancellor, Pattarini et al., 2020). This condition is uncommon as an isolated syndrome on Earth, usually being associated with cancer, or the insertion of a central venous catheter, or hormone induced ovarian hyperstimulation. A recent retrospective study, however, confirmed the safety of oral contraception with respect to potential increased risk of venous thromboembolism in female astronauts (Jain, Ploutz-Snyder et al., 2020). Pharmacological treatment of the internal jugular thrombosis was initiated with enoxaparin, which was immediately available as part of the ISS drug's formulary, followed by apixaban delivered to the station 42 days after the initial diagnosis (Auñón-Chancellor, Pattarini et al., 2020). The need to investigate in detail the effects of relevant drugs under space conditions, outlined since 1983, remains an important issue in space medicine, particularly in the present times with long lasting human spaceflight missions beyond the low Earth orbit (LEO) already planned by Space Agencies and private corporations. Continuous efforts have been made by Space Agencies to develop tracking systems of crew's intake of medications and to standardize the reporting procedures allowing for accurate data collection and monitoring of drug use by astronauts during space missions (Wotring &

Smith, 2020). Inflight data are relevant to understand critical differences between the way drugs act in space in comparison to the Earth (Blue, Bayuse et al., 2019b).

We carried out an extensive literature review on the topic 'physiolo*gical changes that impact drug PK*' with the aims: i) to describe the impact of the space environmental stressors on human physiology in relation to PK (Section 2); ii) to provide the state of the art on experimental data, through the identification of drugs whose PK was studied either in space and/or in ground-based models (Section 3); *iii*) to summarize data on the validation of ground-based models for PK studies (Section 3); and *iv*) to identify possible experimental and research gaps (Section 4).

2. Impact of space environment on human physiology in relation to drug PK

Various physiological changes occur during spaceflight mainly in two distinct phases. During the early phase (72-96 hours), symptoms such as SMS, sleep disturbances, nasal congestion, headache and back pain are reported (Dijk, Neri et al., 2001; Lakin, Stevens et al., 2007). Changes in bone and muscle mass and central nervous system function develop later during the adaptive phase in a time span from weeks to months (Demertzi, Van Ombergen et al., 2016). We also have to consider that some physiological alterations are partially or completely reversible after astronaut's return to the Earth, mainly as a consequence of the cardiovascular system adaptation to weightlessness.

Following is a description of the main organs and systems whose alterations can change Absorption, Distribution, Metabolism and Excretion (ADME) of drugs. Fig. 1 depicts those adaptations and how they influence the single aspects of ADME.

2.1 Gastrointestinal tract and drug absorption

Changes in gastric emptying and intestinal transit time due to SMS may affect the absorption of oral drugs, with significant differences compared to what occurs on Earth. Gastric emptying is slower and more variable in microgravity, in part due to loss of gravity-dependent size and density effects of ingested matter (Amidon, DeBrincat et al., 1991). Intestinal transit rate to the small intestine is instead faster and again more variable. These changes can affect the dissolution rate of tablets/capsules and the stability of drugs in the stomach.

Although it is not known if motility is directly affected by microgravity, gastrointestinal (GI) transit time was certainly affected by SMS treatments (mainly scopolamine and promethazine, given through different routes of administration (Box 1) that were used during spaceflights to counteract the activation of the muscarinic receptor system (Wood, Wood et al., 1987; Wood, Stewart et al., 1990; Davis, Jennings et al., 1993a,b). Weakly acidic drugs may show increased absorption as their permanence in the stomach is prolonged, but for most medications, particularly basic ones, absorption occurs more rapidly in the more basic regions of the intestine. Treatment with antimuscarinic drugs will delay drug absorption, or even reduce it, due to degradation in the stomach induced by the increased residence time in a strong acidic environment, as a consequence of delayed gastric emptying. This altered absorption capability could last for many hours depending on the dose given.

It is not excluded that alterations in gastric pH and expression of epithelial transport systems together with altered blood flow described in hypoxia due to high altitude (Bailey, Stacey et al., 2018; Zhou, Nian, et al., 2018) may occur also in spaceflight.

The presence of food in the gut affects absorption of orally administered drugs through several mechanisms. Specific foods can affect the rate of gastric emptying, and the quality of food in space (e.g. ingredients and consistency) is not exactly the same as on Earth (https://www.nasa.gov/content/space-food-systems). Changes in nutrition, reduced or inadequate caloric intake, alterations in nutrient's ingestion, together with several other stressors associated with space missions, may significantly impact the composition and function of the gut microbiota (for a recent review, please refer to Turroni, Magnani et al., 2020). In addition, absorption at intestinal level or in other organs is affected by the general re-distribution of fluids, which is responsible for tissue oedema (see the section on cardiovascular system adaptations). Additional factors affecting drug absorption include changes in the expression and activity of epithelial and intraluminal enzymes and of transluminal transport systems in the intestine, also due to hypoxia. As far as metabolism is concerned, alterations in the composition of intestinal microflora, and differences in hepatic blood flow velocity and first-pass metabolism will also play a role in drug absorption. In experimental studies under microgravity conditions, changes in the expression profile of multiple proteins in Caco-2 cells, a cell line used as a model to evaluate drug permeability, and therefore the absorption of drugs through the intestinal wall, have recently been reported (La Barbera, Capriotti et al., 2017). Furthermore, decreased activity of lipid-hydrolysing enzymes, activation of proteolytic enzymes and impaired hepatic secretion of the biliary lipid complex have been reported in spaceflight (Smirnow, 1986).

The alterations in gut microbiota can contribute to these events both for the quality and quantity of the faecal mass, and for the spectrum of enzymatic activities involved in the gastrointestinal metabolism and elimination of the xenobiotics. Evidence exists that the predominant species of the gut microflora shift during spaceflight, that is, some species may decrease in number while others increase (Shilov, Lizko et al., 1971). Concerning the intestinal microflora, decrease in "beneficial" Bifidobacterium and Lactobacilli and increase in potentially pathogenic Enterobacteria and Clostridia have been reported (Smirnov, 1986). In recent years, the Astronauts' Microbiome project has been specifically set to assess the impact of long-duration space missions on the human microbiome. The study included 9 astronauts who were required to take samples from different tissues, such as the skin, the nose, the tongue and the GI tract (i.e., stool samples) and at different times (pre-flight, inflight and post-flight) (Voorhies, Mark Ott, C. et al., 2019). The study showed that the composition of the human microbiome was essentially different in different sites, and that samples tended to cluster among sites, as for example the skin and the nose. Microgravity changed the composition of the human microbiome and a greater similarity was observed among astronauts in space in comparison to terrestrial evaluations. Particularly, an increase in alpha diversity and richness was detected inflight in the GI in all astronauts except one. Changes appeared reversible after return to the Earth. Consistently, changes in the human microbiome composition were reported during missions in the GI tract, with reduction in *Akkermansia* and *Ruminococcus* genera (5-fold) and in *Pseudobutyrivibrio* and *Fusicatenibacter* (3-fold) (Voorhies, Mark Ott, C. et al., 2019). Interestingly, *Akkermansia* is endowed with anti-inflammatory properties, therefore the observed reduction may contribute to increased immune inflammation occurring in space (Turroni, Magnani et al., 2020). Consistently, changes in microbiome composition were associated with modification in the blood levels of pro-inflammatory cytokines (increased concentrations of interleukin 1β, IL-1β; tumour necrosis factor α, TNFα; IL-2; monocyte chemoattractant protein-1, MCP-1; macrophage Inflammatory protein-1 beta, MIP-1β) and anti-inflammatory mediators (increased levels of IL-1

receptor antagonist, IL-1ra), particularly at day 180 in space (Voorhies, Mark Ott, C. et al., 2019). In general, it is clear that microflora can affect drug absorption (Fleisher, Li et al., 1999; Schneeman, 2002); however, it has not yet been determined whether the changes that have been seen during spaceflight significantly affect medicine's absorption. Human microbiome research is therefore an essential topic in the agenda of Space Agencies, especially in consideration of long-duration human space missions beyond LEO. These data will enable the adoption of effective countermeasures to ensure safety and health of the crewmembers during these high-risk expeditions (LaPelusa, Donoviel et al., 2021).

All the above features contribute to the so-called "bioavailability" of a drug, which is defined as the ratio of the concentration of the drug available in the circulation after a specific route of administration (whether oral, subcutaneous or intramuscular) and the one after intravenous administration of the same amount of drug. While bioavailability of orally given drugs changes in spaceflight, the one associated with intravenous, intramuscular, rectal and intranasal administered drugs is similar to or better than that on Earth. Modifications of a drug's bioavailability can cause profound changes in the onset, duration and magnitude of the expected effects (Box 1). To date, limited data are available on the overall effects of this complex interplay of factors on the efficacy and safety of the most common drugs. Acetaminophen appears to be the drug for which more PK data in spaceflight have been collected and reported in the literature, starting from the pioneer data by Cintrón, Putcha et al. (1987a).

2.2 Cardiovascular system and drug distribution

The physiological gradients of arterial, venous and microcirculatory pressure are no longer present in microgravity, which causes a shift of fluid from the lower to the upper part of the body and a decrease in blood volume (Charles & Bungo, 1991; Charles & Lathers, 1991; Leach, Inners et al., 1991a; Montgomery, Parmet et al., 1993). This headward fluid shift distends the central vasculature containing the primary sensors for the cardiovascular system (*i.e.*, stretch receptors, baroreceptors, and volume receptors) (Strollo, Norsk et al., 1998). Mild hypoxia can aggravate the condition by causing vasogenic brain swelling and oedema as described at high altitude (Lafuente, Bermudez, et al., 2016; Turner and Gattener et al., 2021). The expanded central volume is detected as a "fluid-volume overload." Thus, the body responds by increasing natriuresis and diuresis, decreasing thirst, and increasing evaporation through the lungs and the skin (Natochin, Kozyrevskaya et al., 1975; Leach, Johnson et al. 1988; Leach, Alfrey et al., 1996; Charles & Bungo, 1991; Charles & Lathers, 1991). However, further studies report that the baroreceptor-kidney loop does not participate in fluid volume regulation in microgravity the way it does on Earth (Hargens & Richardson, 2009). Beside these conflicting results and hypotheses, the final results are decreases in plasma volume and an overall fluid deficit.

Concerning drug distribution, total body water has been reported to be decreased by approximately 3% after long permanence in space (Leach, 1981). Total body mass declines during spaceflight as lean body mass. Thus, for a given dosage, drug concentration in the bloodstream is expected to be higher. The last data document that the extent of chronic dehydration typically seen in spaceflight is about 1 to 2 % of body mass, with only transient increases at launch. Fluid redistribution is not only related to the whole cardiovascular system, but also to extra- and intracellular water movements. One study suggests that the volume of intracellular fluid increases (Leach, Alfrey et al., 1996). In

addition, other available data would confirm that a redistribution of fluids occurs during spaceflight (from plasma volume to the extracellular compartment, from the extracellular to the intracellular volume) more than a loss of total body water due to dehydration or increased diuresis. Other blood components (red cells and proteins, like albumin) also reduce in a few days, so that final blood concentrations return to normal, but overall blood volume is a little lower (Smith, Lane et al., 2019). The drug apparent distribution volume (Vd) would thus be lower, and effective drug concentrations would then be all higher.

There are also other cardiovascular changes that occur during spaceflight, such as decreased left ventricular end-diastolic volume and stroke volume indexes, with compensatory increased accelerated heart rate for the maintenance of cardiac output (Mulvagh, Charles et al., 1991). All these alterations bring to the phenomenon of cardiovascular deconditioning (Antonutto & di Prampero, 2003). In addition, non fatal cardiac arrhythmias have been reported during space missions, with increased risk during longer explorations. Several factors may trigger cardiac rhythm's alterations, including prolongation of the QT interval, hypokalaemia, exposure to radiations leading to myocardial damage, and psychological stressors (Anzai, Frey et al., 2014). Fluctuations in cardiovascular parameters can affect the PK of drugs, thus affecting their safety and effectiveness. However, these cardiovascular changes undergo adaptation after few days of spaceflight, and crewmembers typically do not report long-term fluid problems, since the body seems to adapt to this new condition (Hargens & Watenpaugh, 1996). The problem of new fluctuations of these phenomena however manifests upon return to Earth. Taken together, these results provide support for a model that includes a fluid shift on flight day 1-2, upward in the body and from the plasma, interstitial, and extracellular spaces into the intracellular spaces. There is no convincing evidence regarding the distribution of drugs during or after any fluid shifts. No additional distribution evidence from in-flight studies has been described after publication of V.E. Wotring's report (Wotring, 2011).

Drug binding to plasma proteins, lipids and erythrocytes is probably altered, but only a few direct studies on these parameters have been performed. Plasma albumin and HDL cholesterol are known to be decreased in spaceflight. As a consequence, the percentage of circulating free drug is higher, thus leading to increased availability of the drug for its target(s), accelerated clearance, but also to possible worsening of adverse effects. Concerning hematologic indices, results are contrasting, due to different time-points for blood sampling. Red blood cell mass (haemoglobin and number of erythrocytes), but not haematocrit (Tavassoli, 1982; Leonard, Leach et al., 1983; Leach & Johnson, 1984; Grigoriev, Bugrov et al., 1991) have been reported to be reduced after short duration spaceflights. Erythropoietin levels have been found decreased throughout the flight (Alfrey, Udden et al., 1996a,b), which could be the cause of the increased neocytolysis (i.e. destruction of young red blood cells) (Trial, Rice et al., 2001). On the contrary, red blood cells and haemoglobin were reported to be elevated during long-term spaceflights (Kunz, Quiriarte et al., 2017). It is thus mandatory to evaluate the correlation among hematologic parameters and altered free drug concentration during long permanence in space, since they change during the different mission phases.

Endothelial cells are very sensitive to the absence of gravity (Morbidelli, Monici et al., 2005), therefore a state of endothelial dysfunction can result, accompanied by vascular oxidative stress and a mild chronic inflammatory state (Kapitanova, Muid et al., 2012; Maier, Cialdai et al., 2015). Spaceflight induced disturbance of the brood brain barrier (Mao and Nishiyama et al., 2020), due to

chronic mild inflammation and redox unbalance, can be responsible for brain impermeable drugs to enter the brain, thus causing toxic adverse effect. No specific information on the functions of the transport system in the absence of gravity has been reported for the microvascular and/or lymphatic endothelium, as well as for the permeability status of the endothelium within the organism, both phenomena being involved in the absorption and distribution of drugs to and through the body tissues.

In space, there is no compression of peripheral vessels, and the peripheral hemodynamic performance does not favour tissue perfusion (Regnard, Heer et al., 2001). During spaceflights of long duration, there is a loss of bone and muscle mass, as well as of muscle strength. Drug tissue binding may be altered as a result of massive protein loss (Leonard, Leach et al., 1983) and scarce perfusion. Although an intensive physical training plan can effectively reduce bone loss (Iwamoto, Takeda et al., 2005; Hargens, Bhattacharya et al., 2013), reduced muscle mass can affect the distribution and storage of drugs within this tissue. Again, no direct studies on this topic are present in the literature.

2.3 Drug Metabolism

Cytochromes P450 constitute the largest family of phase I enzymes responsible for the metabolism of drugs and xenobiotics. Cytochrome variations can lead to an increased or decreased metabolism, as well as a different profile of drug and xenobiotic metabolites. All this could result in the appearance of unwanted pharmacological effects or therapeutic failure. Significant changes in hepatic content of metabolic enzymes belonging to the cytochrome P450 family and P-glycoprotein have been described in experimental animals maintained under microgravity conditions (Merril, Hoel et al., 1990; Lu, Bai et al., 2002; Moskaleva, Moysa et al., 2015). In rats flown to space for 14 days, morphological analysis showed that hepatocytes were larger than those of control animals, although the livers themselves were not larger (Racine & Cormier, 1992). In rats flown on Spacelab 3 (for 7 days), a decrease of ~50% was seen in total cytochrome P450 enzymatic activity, whereas no change occurred in the Phase II enzyme glutathione S-transferase (Hargrove & Jones, 1985). In rats, after an 8-day flight on STS-63, a reduction in the amount of the liver enzymes catalase and glutathione reductase (both involved in general antioxidant activity), as well as glutathione (GSH) sulphur-transferase was found (Hollander, Gore et al., 1998). Activation of lipotoxic pathway has been demonstrated in mice after a 13-day spaceflight mission (Jonscher, Alfonso-Garcia et al., 2016). This suggests a progressive liver damage and a predisposition to non-alcoholic fatty liver disease. It is however not known if protein concentration or amount correlates with enzymatic activity in these conditions.

The decreased hepatic metabolism during spaceflight is consistent with the decreased hepatic blood flow due to hypovolemia. Nevertheless, conflicting data have been reported in the literature. Hepatic blood flow has been found to be increased in spaceflight, hypothesizing that more drug is delivered to the liver and processed by first-pass metabolism, thus reducing its circulatory level (Saivin, Pavy-Le Traon et al., 1995). At the same time, a slight increase in liver size and liver filling has been reported after 9 months of spaceflight (Grigoriev, Bugrov et al., 1991).

Hypoxic environment can influence xenobiotic metabolism. Human studies on high altitude hypoxia indicated that the metabolism of most drugs is reduced (Bailey, Stacey et al., 2018). In particular CYP-450 monooxygenase activity can diminish since O_2 is a pivotal substrate. Recently, changes in

the pharmacokinetics of acetaminophen and metformin hydrochloride have been observed in rats under simulated high altitude hypoxia conditions. These modifications were driven by a significant decrease in the transcription of uridine diphosphate glucuronyltransferase 1A1 (UGT1A1) and organic cation transporter 2 (OCT2) (Zhu, Yang et al., 2021).

A fine control of either the major liver enzymes or the levels of circulating metabolites of drugs with low therapeutic index is therefore mandatory for the definition of safe and effective therapy. The innovative approach of metabolomics could meet this need. NASA's GeneLab database (https://genelab.nasa.gov/) will support this medical need by collecting and providing access to the genomic, transcriptomic, proteomic and metabolomic data from spaceflight studies (Berrios, Galazka et al., 2021).

In conclusion, metabolic enzyme systems are not equally affected by spaceflight. More detailed experiments on these identified genes and enzymes should be performed, especially those involving the enzymes that metabolize the drugs used in spaceflight. Interestingly, approximately 31% of all drugs in the ISS pharmacy are metabolized by polymorphic liver enzymes, which can significantly contribute to variability in drug PK, efficacy, and safety (Stingl, Welker et al., 2015). On top of this, data on drug-drug, drug-diet, and drug-physical therapy interactions in the space environment are also lacking (Berman & Eyal, 2019).

2.4 Excretion of drugs

Changes in organ perfusion (kidneys) and in renal function are reported, with an influence on the parameters related to the secretion and elimination of drugs and/or their metabolites. In space, there is a decreased urinary excretion secondary to blood volume contraction. Since all drug-binding macromolecules in the blood are decreased, the drug free fraction is increased, which increases its renal clearance. However, renal plasma flow (RPF), glomerular filtration rate (GFR), and urine production were shown to be unchanged in space (Drummer, Heer et al., 1993; Drummer, Gerzer et al., 2000a; Drummer, Hesse et al., 2000b), contrary to previous data from simulated microgravity models that suggested increased GFR and diuresis (Norsk, Drummer et al., 2001). Instead of increased natriuresis, an increased sodium reabsorption was observed in flight (Norsk, Christensen et al., 2000), resulting in a positive sodium balance. In addition, under hypoxia altered capillary pressure and urinary epithelial biochemistry and transport carrier expression and function may be responsible for increased half-life time ($t_{1/2}$) and area under the drug-time curve (AUC), and reduced clearance rate (Cl) found at high altitude (Bailey, Stacey, et al., 2018). It is not excluded that this happens also in space environment.

Direct studies on renal blood flow are absent from the literature since the D-2 Spacelab mission (Kuipers, 1996). In-flight measurements indicate a slight reduction in total body water for the first few days of spaceflight (Leach, Inners et al., 1991a). There is also the indication that in weightlessness, fluid moves from the blood to the tissues, probably caused by the decrease in the mechanical pressures over tissues and organs (Leach, Alfrey et al., 1996). Abrupt cessation of large muscle group activity may also contribute to decreased plasma volume (Christensen, Drummer et al., 2001). This would be expected to reduce renal blood flow and drug excretion. Indeed, plasma renin activity and antidiuretic hormones were increased in short-duration spaceflights (Leach, Cintrón et al., 1991b; Leach, 1991c). Reduced intake of fluids and fresh food has been proposed as an explanation for reduction in plasma volume (Norsk, 2005). A search of the literature shows no

studies reporting on drug excretion in microgravity or during spaceflight (see further details in the present review). Being able to have simple and immediate systems to check the elimination of drugs and/or their metabolites in the urine could help to optimize the dosage of drugs in space.

3. Drug PK studies in space and surrogate models of microgravity

Important physiological adjustments, as outlined in Section 2, occur during spaceflight with a potential impact on drug PK, thus also on efficacy and safety of medicines in space. Studies that have investigated drug PK directly in spaceflight or in surrogate experimental models (*i.e.*, bed rest, BR) were identified through a search on the PubMed database (accessed on May 08, 2021), as detailed in Fig. 2A. A total of 43 papers were selected for this review. Among them, there were 24 reviews and 2 editorials, which account for 60.5% of the selected publications, 4 inflight studies and 13 research studies based on ground-based experimental models of microgravity (Fig. 2A). As shown in Fig. 2B, the majority of studies, that is 33/43 (77%), were published between years 1986 and 2007. No original articles were published in the last 9 years concerning drug PK in human spaceflight. The most commonly used experimental model of microgravity for PK studies is the bed-rest model, either in the horizontal position (6 articles out of 13) or with the head tilted down (additional 7 articles out of 13).

Based on published original articles, we provide in the following section information on: *i*) drugs whose PK was studied either in spaceflight and/or in ground-based models; *ii*) data on validation of ground-based models for PK studies. Data are presented according to the different experimental conditions adopted.

3.1 In-flight observations.

In-flight information on drug PK is mostly derived from crewmembers involved in the Space Shuttle Flight program. As shown in Table 1, there were 4 publications that reported drug PK data obtained inflight, including 1 study on oral scop/dex and 3 studies on oral acetaminophen (Table 1). The PK of oral scop/dex (0.4 mg/5 mg) was investigated in-flight in 3 crewmembers involved in two different Space Shuttle missions. This study also tested the feasibility of performing PK studies in space using saliva samples. First, it was determined a saliva/plasma ratio in healthy volunteers on the ground. This ratio was found to be constant along the entire scopolamine disposition profile after both intravenous and oral administration, although data were not shown. Then PK data for scopolamine were obtained in space using saliva specimens, whereas no measurements of dextroamphetamine concentrations were reported (Cintrón, Putcha et al., 1987b). Significant interindividual variability in the PK of scopolamine was observed both on Earth and in-flight. In one astronaut, a significant reduction of scopolamine peak concentration (C_{max}) with a parallel increase in the time to peak concentration (T_{max}) was observed in space in comparison to the PK profile assessed on the ground. In a second crewmember, increased C_{max} with unmodified T_{max} was observed. The PK profile of scopolamine was unusual in this astronaut, with C_{max} plateauing for a few hours. In the third crewmember, an erratic PK profile was determined at mission day 0-1, with two peak concentrations, whereas at mission day 2-3, increased C_{max} and slightly decreased T_{max} were found. Notably, drug PK profiles lacked several time points due to inadequate sampling (Cintrón, Putcha et al., 1987b). These data therefore underlie the difficulties of performing drug PK studies in space. Variability in drug PK may explain the reduced efficacy of oral scop/dex observed inflight (Davis, Jennings et al, 1993b). As reported in this study, oral scop/dex in-flight was largely ineffective. In

fact, 9 out of the 19 crewmembers taking scop/dex to treat SMS remained symptomatic on the second day of flight or later. In addition, 7 out of 19 scop/dex users developed symptoms while on medication. Only 3 out of 19 scop/dex users were asymptomatic (Davis, Jennings et al, 1993b). Interestingly, none of the 15 crewmembers that were treated with intramuscular promethazine had more than mild SMS on the next day of flight (Davis, Jennings et al, 1993b). Further information on the efficacy of promethazine for symptomatic treatment of SMS is reported in Box 1.

The PK of acetaminophen was also studied in-flight using saliva samples in a study involving 5 different astronauts participating in 3 different Space Shuttle missions. The study confirmed higher variability of acetaminophen PK in-flight *vs* ground. Significant changes in the absorption phase were reported, with increased C_{max} and reduced T_{max} in 2 subjects with sampling done at mission day 2. Opposite effects were observed at mission day 4, when physiological adaptation to weightless conditions reached an equilibrium. In one subject both C_{max} and T_{max} increased, and an erratic PK profile was detected at mission day 3. This crewmember experienced severe SMS symptoms, which possibly contributed to the abnormal salivary concentrations observed (Cintrón, Putcha et al., 1987a). In a subsequent report including data from 12 subjects on 7 different flights, after oral administration of 650 mg of acetaminophen, an increase in T_{max} on mission day 0 *vs* pre-flight measurements was shown. Consistent with previous evidence, salivary concentrations over time were highly variable in the same subject on different flight days. However, C_{max} tended to decrease on mission day 0 and increase on mission day 2 and 3, while T_{max} tended to increase (Putcha & Cintrón, 1991). A third PK study of acetaminophen was carried out including 10 healthy crewmembers involved in ISS expeditions. Participants were divided into two parallel groups of 5 men. Two different formulations of acetaminophen were tested. The first group, with mean age of 44.6 years (range from 39 to 50 years), received 500 mg of acetaminophen in tablets, whereas the second group, with mean age of 44.4 years (range from 40 to 47 years), received the same dose in capsules. PK was studied in-flight and two months before the space mission under usual living conditions. On Earth, a delay in the rate of acetaminophen absorption was observed when the drug was administered in tablets, without any significant changes in drug bioavailability observed among the two formulations. The PK curves were practically identical during the elimination phase for both formulations when the PK was studied on Earth. On the other hand, in-flight PK data indicated that drug absorption was delayed after the administration of tablets in comparison to what occurred on Earth. Moreover, two peak concentrations were detected. When given in tablets, bioavailability tended to increase in space in comparison to the Earth. For the encapsulated formulation, decreased T_{max} was observed in space in comparison to the terrestrial evaluations. Other PK parameters, including elimination $t_{1/2}$, retention time and Vd were significantly increased. No significant changes in drug bioavailability were reported (Kovachevich, Kondratenko et al., 2009). Based on these results, the authors concluded that encapsulated acetaminophen was preferred to the tablet form in space.

3.2 The horizontal bed-rest model

The horizontal bed-rest (HBR) model was initially used as a physiological analogue of spaceflight to investigate the effect of prolonged exposure to microgravity in human subjects (Hargens & Vico, 2016). The recumbent position produces 0 Gz force on the human body (Watenpaugh, 2016). A study published in 1994 showed that similar cardiovascular changes occur during HBR (0° head-down tilt, HDT) and after spaceflights of similar duration, providing a direct validation of the model,

although only related to cardiovascular adaptiations (Moore, Charles et al., 1994). As shown in Table 2, six PK studies were carried out in the HBR between years 1976 and 1992, thus before the actual validation of the experimental model. All studies were performed according to a crossover design and included between 6 and 12 healthy volunteers. Apart from one study that enrolled subjects with mean age of 50.2 years (Kates, Harapat et al., 1980), all the other studies recruited younger subjects, aged between 20 and 36 years. The gender was specified in five studies enrolling a total of 41 patients, 36 of which were males and 5 females (Elfstrom & Lindgren, 1978; Kates, Harapat et al., 1980; Rumble, Roberts et al., 1986; Rumble, Roberts et al., 1991; Renwick, Ahsan et al., 1992). None of these studies included a direct comparison of drug PK between HBR and spaceflights of similar duration. Three studies focused on antibiotics, whereas others were mostly on anti-inflammatory drugs and pain relievers.

From these studies, it emerges that the absorption of orally administered pivmecillinam is delayed and reduced in the supine position in comparison to the orthostatic position. A slight reduction of drug bioavailability was observed in the supine position, as shown by the mecillinam serum AUC (Andrews, Kendall et al., 1976). No significant differences were detected in mean plasma concentrations, when similar drugs were administered intravenously. This was observed for benzylpenicillin given intravenously at the dose of 600 mg after 1-day HBR (Rumble, Roberts et al., 1986) as well as for penicillin administered intravenously as a rapid bolus at a dose of 1,000,000 U after 6-day HBR (Kates, Harapat et al., 1980). The urinary blood flow appeared to be significantly higher during HBR, but it did not alter the Cl of benzylpenicillin (Rumble, Roberts et al., 1986). In both studies, mean plasma concentrations tended to be lower during HBR in comparison to the orthostatic position, however differences were not significant. In addition, no significant differences in other PK parameters including $t_{1/2}$, Cl, Vd, and AUC were induced by HBR (Kates, Harapat et al., 1980; Rumble, Roberts et al., 1986). From these data, we can hypothesize that the recumbent position may interfere with drug absorption, leaving largely unaltered other physiological functions relevant to drug disposition.

Consistent with this hypothesis, delayed absorption of oral administered acetaminophen was also reported in another study (Rumble, Roberts et al., 1991). However, this effect did not significantly modify drug exposure, as shown by drug AUC. In contrast, absorption of acetaminophen was more rapid in subjects lying on the right side or ambulant in comparison to subjects laying on the left side. However, no relevant changes in other PK parameters were reported (Renwick, Ahsan et al., 1992). In this study, subjects were given 1 g of acetaminophen and 2 x 10 mg of nifedipine during three different visits, after overnight fasting. At each visit, subjects were randomly requested to maintain one specific posture for 4 hours, including lay down on the right and on the left side, and standing position (Renwick, Ahsan et al., 1992). Similarly, the absorption of orally administered nifedipine was more rapid in ambulant subjects and subjects lying on the right side in comparison to the left side. Moreover, the C_{max} and AUC of nifedipine were significantly increased in subjects lying on the right side or standing in comparison to left side recumbent position (Renwick, Ahsan et al., 1992). It should be noted that PK data are commonly derived from subjects adopting a supine position during the initial period of assessment of orally administered drugs. Gastric emptying is increased when lying on the right position and further enhanced by standing or ambulation. On the other hand, renal blood flow and liver blood flow are higher in recumbent patients, and this can account for more rapid drug elimination. In a study including 6 subjects, it was shown that the elimination rate and Cl of phenazone were increased during bed rest, while Vd was reduced. Conversely, the supine position

did not significantly affect the absorption and bioavailability of phenazone (Elfstrom & Lindgren, 1978). Finally, no significant changes in the Cl of lidocaine were observed during HBR, as well as in other PK parameters (Kates, Harapat et al., 1980).

These HBR studies varied in length and for the standardization of other parameters, including food intake, fasting, and blood sampling. Conflicting results are possibly due to the characteristics of different drugs tested. The main gap is the lack of in-flight validation of HBR data, whereas in other settings, as for example for the evaluation of bone loss due to microgravity comparative data are available (Hargens & Vico, 2016).

3.3 The head-down tilt bed-rest model.

The HDT bed-rest (BR) model, using various angles (see below), has extensively replaced the HBR and it is currently regarded as the model of choice to mimic microgravity on the ground, particularly to investigate cardiac and muscle atrophy, orthostatic intolerance and bone loss due to microgravity and develop specific countermeasures (Hargens & Vico, 2016). Subjective and empirical in-flight observations that fluid shift toward the upper part of the body exceeded that seen with HBR led to this further development. The HDT angles used range from 4° to 15°, but 6° became the most common angle used. This 6° tilt down angle produces approximately -0.1 Gz force on human body (Watenpaugh, 2016). Interestingly, cardiac rhythm alterations have been reported during long-term 6° HDT BR (Caiani, Martin-Yebra et al., 2016), in line with in-flight data. Later, the HDT model has been adapted to study lunar gravity level. A lunar gravity component parallel to the long-axis of the body is achieved by using ~ 9.5° tilt angle (Cavanagh, Rice et al., 2013). As shown in Table 3, the PK of several drugs was investigated by using mostly the 6° HDT BR model, including antibiotics, anaesthetics, pain relievers and anti-motion drugs, with conflicting results.

For example, it was shown that total plasma concentrations of ciprofloxacin were not significantly affected by simulated microgravity, *i.e.,* drug administered after 2-day 6° HDT BR. The PK curves showed slightly reduced C_{max} and increased T_{max} due to 6° HDT BR in comparison to the orthostatic position. Slightly lower muscle tissue penetration of ciprofloxacin was observed in simulated microgravity (Schuck, Grant et al., 2005). This is in line with the hypothesis that tissue perfusion may be altered in space due to lack of mechanical pressure and decreased plasma volume, as summarized in Section 2.2. Another study was carried out to evaluate the PK/PD profile of the anaesthetic propofol in simulated microgravity. Plasma samples were collected during and after anaesthesia, and the therapeutic response of propofol was monitored by the sedation score and the bispectral index, an electro-encephalograph (EEG)-derived measure for the state of anaesthesia (Seubert, 2007). This study showed that 2-day 6° HDT BR caused significant haemoconcentration, including increased haemoglobin, haematocrit, platelet, and white cell blood counts in comparison to orthostatic position. However, despite these physiological changes, no significant effects were observed on bispectral index after intravenous administration of propofol in the dose range of 25-200 µg/kg/min to subjects exposed to 2-day 6° HDT BR. No significant differences in time spent unconscious were observed in comparison to subjects not exposed to bed rest. PK was evaluated in the final 15 min of drug administration at 200 µg/kg/min, a dose that gave 40-50 bispectral index in the previous trial. Propofol plasma concentrations were increased by exposure to 6° HDT bed rest, up to 60 minutes after the anaesthetic withdrawal. However, mean dose delivered was similar in both groups, recovery time was the same and no evidence of delayed postoperative cognitive

dysfunction was detected (Seubert, 2007). These data would therefore suggest that the observed changes in drug PK due to BR did not affect drug efficacy. In contrast, during 6° HDT BR, the AUC of lidocaine, administered intravenously at 1 mg/kg, appeared decreased from 130.69 ± 47.65 mg*min/L on day 1 to 92.51 ± 21.43 mg*min/L on day 5. Subjects were ambulant on day 1, then were exposed to 6° HDT BR between day 2 and 5 and were ambulant on day 7. A total of 8 subjects were enrolled in this study. Breakfast without lipid and juice was provided before drug administration and 200 mL of water was allowed 4 hours after drug administration. Consistently, lidocaine C_{max} was reduced at day 2 *vs* day 1 when subjects were in 6° HDT BR, then stabilized during the following days. No more than 20% difference was observed during day 2 up to day 7. Lidocaine Cl and Vd were increased on day 2 *vs* day 1, then stabilized or returned near to basal level. Drug $T_{1/2}$ regularly decreased between day 1 and 7. However, these differences were not significant due to the variability observed on the first day (Saivin, Pavy-Le Traon et al., 1995).

The PK of acetaminophen was studied in subject exposed to 6° HDT BR for different times, including 1 day, 18 days and 80 days. Subjects were lying on the back for 6 hours after administration then other positions were allowed but always supine. Acetaminophen was orally administered at the dose of 1 g with 200 mL water after overnight fasting. Liquids were allowed 4 hours after drug administration and a full meal was provided 6 hours after administration. A 30% increase of C_{max} was observed after 1 day of 6° HDT BR in comparison to control level measured in ambulant conditions. Consistently, T_{max} was reduced by 44%. A trend to increased AUC and reduced $T_{1/2}$ was also observed. Similar findings were reported after 18 days and 80 days of 6° HDT BR, with differences increasing in parallel to the length of bed rest (Gandia, Bareille et al., 2003). These data therefore suggest that the rate of drug absorption is increased during 6° HDT BR, in contrast to what observed using the HBR (Rumble, Roberts et al., 1991; Renwick, Ahsan et al., 1992). Finally, no relevant differences in PK parameters were observed for orally administered ibuprofen after 1-day HDT BR (angle not-specified) (Idkaidek & Arafat, 2011), whereas 30% increased exposure to promethazine was found after 2-day 6° HDT BR especially when the drug was administered *per os* (Gandia, Saivin et al., 2006).

As outlined for HBR, variability in drug PK observed using the HDT BR model, mostly at -6°, may depend on the characteristics of the drugs and lack of standardization of the studies. In this regard, the NASA Flight Analogs Pojects was specifically set out to standardize the experimental conditions in BR studies, with data on cardiovascular adaptation occurring during long-term 6° HDT BR available in the literature (Platts, Martin et al., 2016). Again, the main gap in PK studies is the lack of inflight validation of 6° HDT BR data.

3.4 Parabolic flight

Parabolic flight is an experimental model aimed to generate alternating periods of free fall (reduced gravity) and high gravitoinertial force level (~1.8-2G), each lasting 20-25 sec. A flight usually consists in 30 consecutive parabolas and motion sickness is scored according to different rating systems (Graybiel & Lackner, 1987; Kohl, 1987). This terrestrial model of microgravity has been used to study the efficacy of different anti-motion sickness medications. However, it seems unsuitable for PK studies. A summary of the evidence on the efficacy of anti-motion drugs evaluated using the parabolic flight model is presented in Box 2.

4. Research gaps in drug related PK studies

Relevant physiological changes occur during spaceflight that may impact drug PK, thus highlighting the need for controlled studies on drug PK in space. However, despite general agreement on this priority, as reported in several review articles published over time, data on drug PK obtained in-flight are still limited to seminal observations dated back to 1987-1993 with only a more recent study carried out on the ISS in 2009. Similarly, it has not yet been determined whether changes in physiological processes occurring by exposure to space environment significantly affect drug PK. This includes for example changes in the expression and functioning of transport system at enteric level or the influence of gut microbiota shift in drug processing and absorption. Moreover, no direct studies on drug distribution, metabolism and elimination are present in the literature. There is no convincing evidence about the distribution of active principles or their metabolites during and after any fluid shift due to spaceflight. Also, the effects of changes in bone and muscle mass and endocrine rearrangements on drug distribution and bioavailability remain to be established. Only sporadic reports are available on the effect of real microgravity on the structure and function of blood and lymphatic endothelium, as cell viability, permeability and exposure of metabolizing enzymes or transport systems, to be put in relation to medicine absorption and distribution in the critical organs. Another important gap regards the changes in haematological parameters, including the plasma protein levels, which can influence the free drug concentration and precipitate unwanted adverse reactions. An extensive and complete study on the liver enzymatic pattern is still lacking, also including the polymorphic character of these metabolic pathways. Drug excretion is a further parameter that merits to be studied in detail due to dated studies and controversial results. On top of this drug-drug interactions and drug-diet components interactions remain to be assessed inflight in order to maximize the efficacy and guarantee the safety of medications.

Spaceflight research in this field can benefit from recent developments in wearable sensors allowing continuous measurements of physiological functions (Li, Dunn et al., 2017). The lack of comprehensive drug PK studies is probably due to the difficulty of carrying out multiple blood-sampling in real microgravity and lack of simple and immediate systems to check the elimination of drugs and/or their metabolites in the urine. The use of alternative specimens for PK studies is limited to saliva and to the study of scopolamine and acetaminophen PK in-flight. A consistent saliva/plasma ratio for these drugs on a range of plasma concentrations and over the disposition profile was established on the ground but no validation in-flight was performed. It is unknown whether the consistent saliva/plasma ratio measured on ground is maintained in space. Therefore, in order to use saliva samples for in-flight PK studies, an evaluation of the correlation between drug concentration in saliva and blood should be performed in space. In addition, ground-based models of microgravity appear insufficiently validated as predictive models of drug PK in space. There is a substantial lack of comparative studies on drug PK between experimental models of microgravity and real microgravity.

5. Conclusions

The use of medicines during human spaceflight is on average increased in comparison to the use of drugs on Earth, as suggested by a recent study in which data on medicine usage on the ISS were collected from six astronauts through an iOS application (Wotring & Smith, 2020). An average of 20.6 ± 8.4 medication entries per subjects (n=5) per flight week was observed, significantly higher than data obtained through medical notes of flight surgeons (Wotring, 2015). However, medicines are currently used in space based on the assumption that they work in a similar manner to what is standardized on the Earth, despite relevant physiological adaptations that occur in space, together

with potential alterations in drug stability due to radiation exposure. The extent of such modifications is currently not well determined, and ground-based models are not sufficiently validated to address these issues. Despite the compelling need to assess how drugs work in spaceflight, available in-flight data are only limited to few seminal studies. For this kind of evaluation, it is mandatory to determine PK parameters for medications frequently used in spaceflight in two different settings: on the Earth and during spaceflights, together with similar studies evaluating drug efficacy in-flight vs. on the Earth. Only with such data we can have a more comprehensive knowledge of pharmacology in space, thus be able to better inform drug prescription. This is particularly relevant for future manned explorations beyond LEO, which will not allow rapid return to the Earth in the event of a medical emergency.

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Figure legends

Fig 1. Schematic representation of the main physiological adaptations to Space environment and their relevance on ADME. The right part of the figure represents a summary of the main alterations described for drug absorption, distribution, metabolism and excretion, resulting from organ and tissue adaptation to unloading, stress, confinement and radiation.

Fig. 2. ***Search strategy and flow diagram followed for the literature review and timeline of the distribution of studies concerning PK in space.*** **(A)** The diagram shows the search strategy adopted to select papers related to PK in space. A total 439 papers were retrieved using 'pharmacokinetics' OR 'pharmacotherapy' AND 'spaceflight' as keywords. An additional 59 studies were identified using 'bed rest' AND 'pharmacokinetics' OR 'drug disposition' as keywords. Based on the abstracts, 36 articles related to drug PK in Space were selected, excluding three articles in Russian for which full texts were not available. Seven studies, reporting inflight data or results obtained via ground-based models, were selected through recent review articles (Kast, Yu et al., 2017; Eyal & Derendorf, 2019), for a total of 43 papers. Among them, there were 24 reviews and 2 editorials, 4 inflight studies and 13 research studies based on ground-based experimental models of microgravity. **(B)** The graph reports the number of papers published per year, related to drug PK in Space. Articles were grouped in Editorials, Reviews and Original papers. The latter were divided in publications reporting inflight data or results from ground-based models of microgravity, including 6 publications using the supine bed rest model and 7 studies employing the head tilt down bed rest model.

Table 1. List of drugs investigated in spaceflights.

ATC Code	Drug	Administration route	Dose (mg)	Subjects (n)	References
A04AD01/ N06BA02	Scopolamine/ Dextroamphetamine	os	0.4/2.5-5	3	Cintrón, Putcha et al., 1987b
N02BE01	Acetaminophen	os	500-650 (2x325)	5 12 10	Cintrón, Putcha et al., 1987a; Putcha & Cintrón, 1991; Kovachevich, Kondratenko et al., 2009

Table 2. List of drugs investigated using the bed rest experimental model, in the horizontal/supine position.

ATC Code	Drug	Administration route	Dose (mg)	Subjects (n)	PK results		References
J01CA08	Pivmecillinam	os	200x2	6	↑ T_{max}; ↓C_{max}; ↓AUC (supine vs ambulant)		Andrews, Kendall et al., 1976
J01CE01	Benzylpenicillin	i.v.	600	7[a]	No significant differences (1 day BR)		Rumble, Roberts et al., 1986
J01CE02	Penicillin	i.v.	1,000,000 U	12[b]	No significant differences (6 day BR); ↑ urinary blood flow, but no effect on drug Cl.		Kates, Harapat et al., 1980
N01BB02	Lidocaine	i.v. over 15 min	100		No significant differences in PK parameters		Kates, Harapat et al., 1980
N02BB01	Phenazone	os i.v	10/kg (gelatine caps) 10/kg	6[b]	No significant differences in PK parameters	↑ elimination rate constant ↑ Cl ↓ Vd	Elfstrom & Lindgren, 1978
N02BE01	Acetaminophen	os	500	8[b]	↑ T_{max}; no difference in AUC		Rumble, Roberts et al., 1991
N02BE01	Acetaminophen	os	1000	8[c]	↓ T_{max} ambulant and right vs left		Renwick, Ahsan et al., 1992
C08CA05	Nifedipine	os	2 x 10		↓ T_{max} ambulant and right vs left; ↑ C_{max} and ↑AUC		Renwick, Ahsan et al., 1992

Notes:

[a]= 4 males and 3 females enrolled.

[b]= all males enrolled.

[c]= 6 males and 2 females enrolled.

Abbreviations: AUC, area under the curve; BR, bed rest; Cl, clearance; C_{max}, maximum concentration; i.v., intravenous administration; os, oral administration; PK, pharmacokinetics; T_{max}, time to maximum concentration; Vd, distribution volume;

Table 3. List of drugs investigated using the head tilt down bed rest experimental model.

ATC Code	Drug	Administration route	Dose (mg)	Subjects (n)	PK results	References
J01MA02	Ciprofloxacin	os	250 mg	6[a]	Total plasma concentration not affected (2 day HDT-BR); ↓muscle tissue penetration	Schuck, Grant et al., 2005
N01AX10	Propofol (2, 6-diisopropylphenol)	i.v. (15 min)	25, 50, 100 and 200 µg/kg/min	20[b]	↑ plasma concentration (2 day HDT-BR). Similar efficacy. Mean dose delivered was similar.	Seubert, 2007
N01BB02	Lidocaine	i.v.	1 mg/kg	8[c]	↓ AUC from day 1 to day 5 ↓ C_{max} day 2 vs day 1, then <20% difference ↑ Cl day 2 vs day 1, then stable ↓ $t_{1/2}$ day 2 to day 7 High variability/not significant	Saivin, Pavy-Le Traon et al., 1995
N02BE01	Acetaminophen	os	1 g (200 mL water)	18[c]	↓ 44% T_{max} (day 1); ↑30% C_{max}; ↑AUC; ↓ $t_{1/2}$ (not significant). Similar changes at day 18 and 80. Opposite results in comparison to supine BR	Gandia, Bareille et al., 2003
M01AE01	Ibuprofen	os	600 mg	6[c]	No relevant differences after 1 day	Idkaidek & Arafat, 2011
R06AD02	Promethazine	os i.m.	25 mg 50 mg	12[c]	↑ 30% exposure (especially per os)	Gandia, Saivin et al., 2006

Notes:

[a]= 5 males and 1 female enrolled.

[b]= 10 males and 10 females enrolled.

[c]= all males enrolled.

Abbreviations: AUC, area under the curve; Cl, clearance; C_{max}, maximum concentration; HDT-BR, head tilted down bed rest; i.m., intramuscular administration; i.v., intravenous administration; os, oral administration; PK, pharmacokinetics; $t_{1/2}$, half-life; T_{max}, time to maximum concentration; Vd, distribution volume;

BOX 1. Inflight use of intramuscular promethazine for the treatment of symptoms of SMS.

Promethazine was first used intramuscularly in March 1989 on a 5 day-long Space Shuttle flight (Kennedy Space Center Historical Report No. 1B, KHR-1B, NASA) for the symptomatic treatment of one male crewmember that developed severe SMS (Bagian, 1991). In this subject, symptoms persisted throughout day 1 and beginning of day 2 of flight despite oral medications. Symptoms completely and persistently resolved half hour after intramuscular administration of a single dose of 50 mg promethazine. Since then up to July 1991, 28 out of 29 astronauts were successfully treated with intramuscular promethazine for the relief of symptoms related to SMS. Efficacy of intramuscular promethazine was shown by a retrospective analysis on 20 out of 96 crewmembers at their first spaceflight exposure across the first 44 Space Shuttle flights, 5 to 10 days long. Intramuscular promethazine, at the dose of 25-50 mg and mostly as a single injection, proved to be effective in 18 out of 20 crewmembers, with a reduction of symptoms observed within 1-2 h from treatment. The drug was also effective if administered in suppository formulations. Intramuscular promethazine was well-tolerated with only three crewmembers out of 20 reporting drowsiness after the injection (Davis, Jennings et al., 1993a). In another study, only one of 15 crewmembers receiving intramuscular promethazine reported drowsiness after injection (Davis, Jennings et al., 1993b). In a subsequent retrospective study on promethazine, only one of the 21 crewmembers studied reported sedation 30 min after the injection. Incidence of sedation was therefore 4.8%, in striking contrast to the 60 to 73% incidence observed in the general population in ground-based studies (Bagian & Ward, 1994). This observation suggests that the PK profile of orally administered drugs may be more variable in Space, in part due to the development of SMS itself. Other routes of administration can possibly reduce such variability and increase drug efficacy. However, no PK studies were carried out in space using intramuscular promethazine that proved this hypothesis. Nevertheless, the drug became standard treatment for SMS on Space Shuttle flights.

BOX 2. Efficacy of anti-motion sickness treatments as emerged by parabolic flights

Scopolamine has been the most investigated drug using the parabolic flight experimental model. The pharmacological effects of scopolamine were evaluated in two different studies according to two different modalities of administration. In a first study including 47 subjects, scopolamine (0.43-0.5 mg) was administered intramuscularly (i.m.) during parabolic flight to severely sick subjects, usually between parabolas 5 and 29. The drug resulted in beneficial effects in 72% of cases (Graybiel & Lackner, 1987). Beneficial effects were considered resolution of motion sickness symptoms and prevention of nausea or vomiting at touchdown. Similarly, promethazine administered i.m. at the dose of 50 mg was effective in 78% of subjects, whereas it was not beneficial at 25 mg (Graybiel & Lackner, 1987). Dramamine administered i.m. at 50 mg did not exert any therapeutic effect (Graybiel & Lackner, 1987). These data would support the hypothesis that the intramuscular administration route can reduce variability in drug absorption due to SMS thus favouring the pharmacological effects of these drugs. However, no PK data are available from parabolic flights. Scopolamine was also effective when administered in a buccal pouch at the dose of 1 mg and maintained in the mouth between parabolas 5 and 30. Therapeutic drug level was estimated to be reached after parabola 10, based on previous investigations on the PK of buccal scopolamine (Norfleet, Degioanni et al., 1992). This study was carried out according to a crossover design, thus subjects were studied in two different parabolic flights and were generally less sick during the second flight. Despite this variability, buccal scopolamine significantly reduced by 31% the severity of nausea and by 50% the total number of parabolas with vomiting (Norfleet, Degioanni et al., 1992). As mentioned above, scopolamine, alone or in combination with dextroamphetamine, has been widely used to treat SMS during Space Shuttle flights. The drug was often dispensed as custom dosage formulation in gelatine capsules alone or in combination with dextroamphetamine. The latter is associated in order to reduce sedative effects of scopolamine. In a PK study, performed on the ground, on the most commonly used formulations of scopolamine during NASA operations, it was shown that drug absorption is delayed when the drug is formulated in gelatine capsules and bioavailability is significantly reduced when the drug is administered in combination with dextroamphetamine (Boyd, Du et al., 2007). This variability may contribute to the lack of efficacy sometimes observed during spaceflights. Finally, oral metoclopramide administered prophylactically 75 mins before parabolic flights did not show any beneficial effects (Kohl, 1987) and it is not used in real microgravity.