In Vitro and *In Vivo* Assessment of Regional Nasal Deposition using Scintigraphy from a Nasal Spray and a Nasal Powder

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SUMMARY

Case studies which utilized scintigraphy to link *in vitro* and *in vivo* nasal spray deposition from a nasal spray and a powder were undertaken. *In vitro* deposition in a nasal cast illustrated that significantly higher deposition ($p<0.05$) was observed in the olfactory region for the unit-dose powder device compared to the multi-dose liquid nasal spray pump. *In vivo* studies in healthy volunteers also demonstrated increased deposition in the olfactory region for the nasal powder compared to the nasal spray.

Coupling *in vitro* and *in vivo* nasal deposition has the potential to provide valuable insight into the utility of *in vitro* studies to inform subsequent clinical studies during the development of nasal drug delivery therapies, whether they be local or systemic.

INTRODUCTION

Nasal drug delivery offers significant opportunities for introducing new therapies, whether they be local, systemic or via the nose-to-brain pathway [1]. Predicting the nasal deposition of new drug candidates in order to facilitate the development pathway is essential for both liquid

and powder formulations. It is important to understand regional nasal deposition as it may be necessary to target different areas in the nasal cavity, e.g., the olfactory region for central nervous system (CNS) therapies. Linking *in vitro* development work and *in vivo* studies can potentially streamline the development process. Historically nasal casts have been used to create *in vitro* nasal deposition data, but these models are known to have limitations or have not been directly correlated to *in vivo* deposition [2, 3], thus limiting their usefulness. This article describes case studies which utilize validated scintigraphy techniques to link *in vitro* and *in vivo* nasal spray deposition from a nasal spray and a powder which could predict subsequent nasal therapy uptake to facilitate the development of nasal drug delivery therapies.

MATERIALS AND METHODS

Nasal delivery devices and formulations

Two different nasal devices and formulations were used to investigate deposition profiles. A multi-dose liquid spray pump (Figure 1A, VP7, Aptar Pharma, France), was used to deliver 50 µL of radiolabelled saline solution per nostril. The radiolabel was Diethylene Triamine Penta acetic Acid – Technicium (DTPA-Tc^{99m}) and one 50 µL dose of the radiolabelled solution had a radioactive content of ~1.5 MBq (megabecquerel). The droplet size distribution was measured by laser diffraction (Spraytec, Malvern Panalytical, UK) following the validated labeling procedure [4] and characterized as Dv10 = 19 µm, Dv50 = 39 µm and Dv90 = 83 µm, respectively.

A unit dose powder device (Figure 1B, UDS powder, Aptar Pharma, France) was used to deliver 10 mg of radiolabelled lactose powder (Respitose®, DFE Pharma, Germany) per nostril. Lactose was radiolabelled with DTPA-Tc^{99m} using a validated labelling procedure [4] in order to deliver ~10 mg of powder per dose with a radioactive content of ~1.5 MBq. The lactose particle size distribution was measured by laser diffraction (Spraytec, Malvern Panalytical, UK) following the validated labelling procedure and characterized as Dv10 = 31 µm, Dv50 = 80 µm, and Dv90 = 190 µm, respectively.

Figure 1. Multi-dose liquid spray pump (A) and unit-dose powder device (B).

In vitro and *in vivo* deposition imaging

In vitro deposition of the nasal spray and nasal powder were characterized in a nasal cast using scintigraphy. The nasal cast model was manufactured from epoxy plastic based on Computed Tomography (CT)-scans of a plastinated head model [5], previously validated as a predictive model for nasal aerosol deposition [6]. Figure 2 shows the four parts of the nasal cavity from the nose to the nasopharynx, allowing the *in-situ* assay of deposited particles in each region of interest. The

nose component was made from silicone to represent the flexibility of the human nostril. Based on Buck *et al*. [7], approximately one third of the upper part of the nasal cavity, between the nose/nasal valve and the rhinopharynx, was considered to be the area that defines the olfactory epithelium, which is often targeted for the nose-to-brain drug delivery pathway.

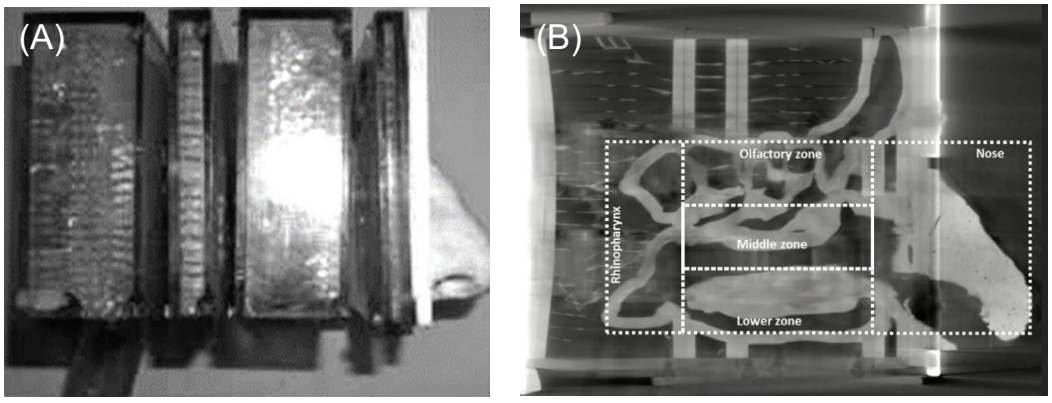

Figure 2. **(A) Nasal cast parts (courtesy Aptar Pharma/DTF/University of Tours) and (B) nasal cast regions of interest. Reproduced with permission from *RDD Europe 2017*, Virginia Commonwealth University and RDD Online [4].**

In vivo deposition imaging was also conducted with six healthy volunteers using the same liquid and powder nasal devices described above, see Figure 1. The liquid and powder formulations were radiolabeled using the same validated procedure that was used in the *in vitro* study [4].

RESULTS AND DISCUSSION

Table 1 compares % deposition in the nasal cast for the nasal spray and the UDS powder device. The radiolabelled images revealed a notable difference between the liquid nasal spray and the UDS powder device in terms of radioactive deposition within the nasal cast. Higher deposition was observed in the olfactory region for the UDS powder device compared to the liquid nasal spray pump.

Table 1.

***In vitro* deposition from a nasal spray and a nasal powder in a nasal cast, n=6. (* p< 0.05).**

Regions of interest	Deposition % +/- SD	Deposition % +/-SD
	Liquid nasal spray	Powder nasal spray
Nose*	51 ± 6	14 ± 4
Lower zone*	13 ± 5	8 ± 3
Middle zone	30 ± 2	37 ± 4
Olfactory zone*	6 ± 3	34 ± 7
Rhinopharynx*	1 ± 0	6 ± 3

Figure 3 compares typical images from the *in vitro* and *in vivo* nasal spray deposition studies. Fiducial markers are the two upper scintigraphy markers visible in Figures 3C and 3D. Stronger scintigraphy signals were noted in the interior and upper parts of the nasal cavity for the powder device whereas more holdup in the nose area was noted for the liquid device.

The radiolabeled images similarly revealed significant ($p<0.05$) differences between the liquid nasal spray and the UDS powder device in terms of radioactive deposition in the nose, lower and olfactory zones. Not surprisingly, increased variability was observed *in vivo* because the volunteers have differing nasal anatomies; *in vitro* studies used a single anatomical model. The liquid nasal spray showed increased deposition in the nose possibly due to a wider spray angle than the powder device (liquid spray angle ~40°, powder spray angle ~20°). More material is able to reach the internal areas of the nasal cavity from the powder device and subsequently increased deposition is observed including the olfactory region (~4 ± 3.8% (liquid) vs ~20 ± 9% (powder)).

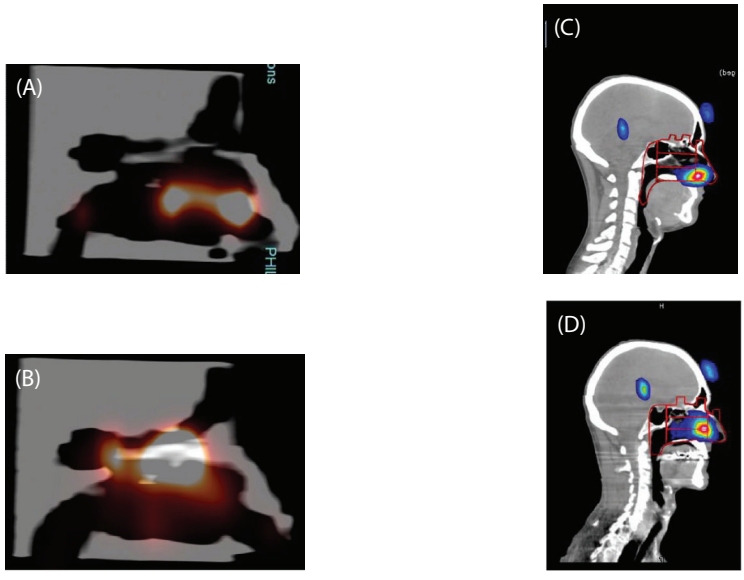

Figure 3. Examples of *in vitro* (left, (A) liquid, (B) powder) and *in vivo* (right, (C) liquid, (D) powder) scintigraphy deposition images.

Both the *in vitro* and *in vivo* studies confirm the same trends with regard to deposition in the nasal cavity from the liquid and powder nasal spray devices. However, correlation of *in vitro* and *in vivo* nasal deposition still remains a challenge due to multiple factors [3]. *In vitro* nasal casts are made from rigid (usually plastic) materials and the internal surfaces do not closely resemble the humid mucosal environment found in the human nasal cavity. This could potentially be mitigated by humidifying and coating the internal surfaces before conducting *in vitro* testing. Device position in the nostril pre-dosing can also influence subsequent deposition *in vitro*; the nasal cast used in this work incorporates a flexible silicone nose-piece which more closely resembles the *in vivo* situation. *In vitro* nasal cast models are often constructed from 3D files of imaging scans from individuals and can show less variability in deposition studies compared to real and diverse patient populations. One way to address this limitation is to validate so-called 'idealized casts' using *in vivo* deposition studies so that the casts are more representative of patients overall. In addition, different *in vitro* nasal casts may need to be developed and validated to cover the various patient populations

that use nasal therapies, e.g., pediatric, geriatric, male, female, etc. Bridging the gap with regards to the potential differences between *in vitro* and *in vivo* nasal deposition techniques should improve *in vitro-in vivo* correlations and bring us closer to useful predictive *in vitro* nasal deposition tools.

CONCLUSIONS

Both *in vitro* and *in vivo* scintigraphy studies confirm the same trends with regards to deposition in the nasal cavity. The UDS powder device delivered a higher dose to the olfactory region and less powder to both the nose and lower regions of the nasal cavity, compared to the nasal spray. Unsurprisingly, it was observed that *in vitro* delivery was less variable than *in vivo* delivery likely due to the fact that the *in vitro* model is based on a single anatomy whereas the *in vivo* work was undertaken with multiple healthy volunteers with differing anatomies.

The outcomes indicate that it is possible to differentiate various nasal spray device parameters including liquid or powder formulations and deposition in nasal regions of interest. Coupling *in vitro* with *in vivo* nasal deposition studies could provide valuable insights into how nasal therapies may be deposited and taken up and provide predictive guidance for pre-clinical efficacy studies. Suitably validated nasal cast models may provide predictive *in vitro* guidance to inform subsequent *in vivo* deposition during the early development stages of nasal drug delivery therapies.

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