Reproducibility of neutron activated Sm-153 oral dose formulations intended for human administration

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ABSTRACT

Neutron activation of Sm-152 offers a method of radiolabeling for the *in vivo* study of oral dose formulations by gamma scintigraphy. Reproducibility measurements are needed to ensure the robustness of clinical studies. 204 enteric-coated guaifenesin core tablets (10 mg of Sm_2O_3) were irradiated by thermal neutrons to achieve 1 MBq at 48 h. Administered activities were 0.86 ± 0.03 MBq. Good reproducibility (CV=3.5%) was observed over 24 weeks ensuring that volunteer doses were within the dose reference level of 0.8 mSv.

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1. Introduction

When designed correctly pharmacoscintigraphy studies performed in conjunction with appropriate *in vitro* testing have proven to be valuable tools for assessing the *in vivo* performance of sophisticated oral dosage formulations under normal physiological conditions in humans (Perkins and Frier, 1996, 2000; Wilding et al., 2001). The use of radioactive tracers to monitor the biodistribution, transit and pharmacokinetics of drug formulations is an attractive concept, especially as the use of gamma camera enables a graphic representation of the distribution and deposition of radiolabeled pharmaceutical compounds (Wilson and Perkins, 1992). An important feature of this methodology is that the quantity of radioactive material required for incorporation into a formulation is very small (a few mg) and if incorporated properly, does not compromise the performance characteristics of the delivery system (Ahrabi et al., 2000; Marvola et al., 2004, 2008). Appropriate *in vitro* testing is necessary to confirm that the incorporation of the radioactive material does not affect the performance of the formulation and to confirm that the biodistribution, transit and pharmacokinetics of the labelled pharmaceutical compounds are representative of those of the unlabelled pharmaceutical compounds.

The most commonly used radioactive tracers in pharmacoscintigraphy are Tc-99m, In-111, Sm-153 and Er-171 (Parr et al., 1985, 1987; Parr and Jay, 1987; Perkins and Frier, 1996, 2000; Wilding et al., 2001). Tc-99m and In-111 have some disadvantages since the radiolabeling requires dedicated radiation protection facilities at the pharmaceutical premises. An additional limitation of Tc-99m is the short physical half-life (6.02 h) with respect to the time needed for manufacturing and imaging especially for the drug delivery to the colon may take up to 12–24 h. Non-radioactive Sm-152 and Er-170 have been introduced in this context to allow formulations to be produced in pharmaceutical facilities and to reduce the radiation exposure to the workers.

Samarium oxide (Sm_2O_3) in micron particle form is recommended for oral drug delivery studies in humans because it is poorly soluble in water and chemically stable hence it will not be absorbed in the gastrointestinal (GI) tract. The natural abundance of Sm-152 is 26.7%. After irradiation with a thermal neutron flux in the range of $1-5 \times 10^{12}$ n cm^{-2} s^{-1}, it produces Sm-153, a gamma emitter with physical half-life of 46.3 h and an intense photopeak energy of 103 keV (28 per 100 disintegration). This gamma energy is well suited for high efficiency, high resolution gamma imaging (Tse et al., 1989).

The radioactivity produced from Sm-153 is dependent on a number of factors including amount of incorporated Sm_2O_3 (mg),

dimension of irradiating product, neutron flux ($cm^{-2} s^{-1}$) and irradiation time (s). In addition the logistics of producing activated dose formulations at a reactor site present challenges for clinical studies especially where ethical approval restricts the administered amount of radioactivity. This study provides essential validation of the neutron activation protocols prior to *in vivo* administration to ensure reproducible radiolabeling with Sm-153 within the administered dose reference limit defined by the protocol and the ethical and regulatory bodies. The formulation validated in this study was enteric-coated guaifenesin core tablet in oblong size 7.0 mm gauge.

2. Materials and methods

2.1. Sm-152 labelled formulation

The studied formulation was an oblong (7.0 mm-gauge) enteric-coated guaifenesin 83 mg core tablet. For each tablet, 0.010 ± 0.001 g of natural abundance samarium oxide (Sm_2O_3 Alfa Aesar, Ward Hill, MA, USA) was blended and mixed together with other materials in the formulation. Tablets weighing 0.97 ± 0.05 g were made on a tablet press (Piccola Model B-10, Riva, Aldershot, Hampshire, UK) using 7.0 mm gauge-tooling. Normal quality control measures included random sampling weighing, sizes measuring and testing for hardness and friability were carried out.

2.2. Neutron activation

The tablets were sent to the reactor at Reactor Institute Delft (University of Delft, Delft, The Netherlands) for neutron activation. The reactor is an open pool-type research reactor utilizing MTR-fuel assemblies and low-enriched U-235 ($< 20\%$) as fuel. The tablets were packed individually in heat sealed plastic blister packs (tubular polyethylene film, A151100, Audion Elektro B.V., The Netherlands) and marked with a unique number. This was to enable individual tablet tracking through the irradiation, calibration and participant dosing procedures. Gloves (Fisherbrand® powder free nitrile gloves) were worn when handling the tablets, as sodium from sweat on the hands could lead to unwanted activation products following irradiation.

For irradiation, six tablets were packed into a polyethylene ampoule approximately 10 cm long and 3 cm in diameter. The ampoule was then transferred to the reactor core by a pneumatic tube transport system. The samples were then irradiated in a thermal neutron flux of $5 \times 10^{12} cm^{-2} s^{-1}$ for 70 s as set by preliminary test protocol to achieve a nominal radioactivity of 1 MBq at 48 h after the irradiation. The thermal to epithermal neutron flux ratio in the facility was 70 whereas the thermal to fast neutron flux ratio was 14. The samples were kept for at least 48 h after irradiation to allow the decay of the unwanted irradiation by-products, primarily Na-24 (Honkanen et al., 2004; Marvola et al., 2004, 2008).

2.3. Dose calibration

Following irradiation the tablets were assayed at the reactor site for radioactive purity using a coaxial, p-type, 40% relative efficiency germanium detector (Princeton Gamma Tech, New Jersey, USA). Gamma spectra were obtained and analysed after corrected for background radiations. The tablets were subjected to further radioactivity measurement at the study site using a radionuclide assay calibrator (CRC-15R, Capintec Inc, New Jersey, USA) at 48 h after the irradiation. This calibrator was pre-calibrated using a Sm-153 standard source from the Reactor Institute Delft prior to the start of the study.

The experimental study required radiolabeled formulations to be produced over a period of 24 weeks to allow the full cross over study to be completed. Dose calibration data were collected to assess the reproducibility of radioactive Sm-153 produced and to ensure that the administered radioactivity did not exceed the limit set by the ethical and UK Department of Health approvals.

3. Results

A total number of 204 tablets were irradiated on 17 separate occasions over a period of 24 weeks. Table 1 summarizes the parameters of the study protocol. The neutron activation protocol was designed to give a radioactivity of 1 MBq from the activated Sm-153 48 h after irradiation. Table 2 shows the result of radioactivity assayed by dose calibrator at 48 h, on receipt at the study site and at 59 h, the time of administration.

The coefficient of variation (CV) for reproducibility of radioactivity produced was 3.5%. Table 3 shows the results of the gamma spectroscopy data that were used to verify the activated by-products from the neutron activation. A plot of the spectrum would only show the peaks from the uranium and thorium series coming from background sources. Analysis of the spectrum after background correction found nothing of interest apart from Sm-153, and a trace amount of short-lived Na-24 with a physical half-life of 14.96 h.

The activities measured at the time of administration for each individual tablet over the 24 weeks were plotted in Fig. 1 to determine if there was any time variation in the activity produced.

Table 1
Summary of the neutron activation protocol.

Amount of Sm_2O_3	0.010 ± 0.001 g
Neutron energy	25 MeV (thermal neutron)
Neutron flux	$5 \times 10^{12} cm^{-2} s^{-1}$
Thermal to epithermal neutron flux ratio	70
Thermal to fast neutron flux ratio	14
Irradiation method	Short irradiation at the reactor core using Pneumatic Transfer System (PTS)
Irradiation time	70 s
Targeted radioactivity approved by ethics committee	1 MBq at 48 h after neutron activation
Schedule for *in vivo* volunteers study	59 h after neutron activation
Estimated effective dose to the volunteer subjects	0.8 m Sv whole body effective dose

Table 2
Results of radioactivity assays using dose calibrator ($n=204$).

	Activity at 48 h following irradiation (MBq)	Activity at time of administration (MBq)
Mean $\pm$ SD	1.01 ± 0.03	0.88 ± 0.03
Max	1.09	0.95
Min	0.91	0.79

Table 3
Gamma spectroscopy data ($n=20$) measured at 11 h following activation.

Radionuclides	Peak energy (keV)	Activity (MBq)
Sm-153	103.18	1.372 ± 0.089
Na-24	1368.6	0.0044 ± 0.0005
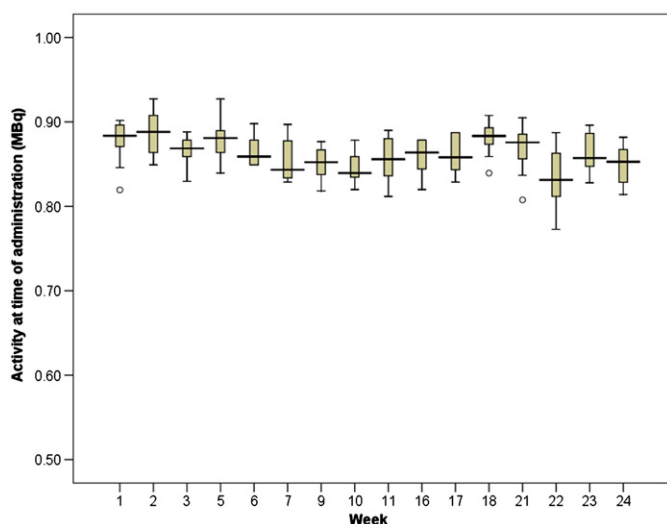
Fig. 1. Box-whisker plot of Sm-153 radioactivity measured for each individual tablet at the time of administration (59 h after neutron activation) over an extended period of 24 weeks. There were 12 tablets irradiated every week except for week 4, 8, 12–15, 19–20, when there was no neutron activation carried out due to bank holidays. The 25th and 75th percentile marks the box and whiskers extend to the range outliers excluded. The median is marked in the box. The open circles (o) are outliers that represent data of more than 1.5 box-lengths from the 75th percentile.

4. Discussion and conclusion

In most countries the administration of radioactive materials to human subjects is controlled by regulatory authorities. In the United Kingdom all research involving the human administration of radioactivity is controlled by the Administration of Radioactive Substances Advisory Committee (ARSAC). When undertaking clinical trials in healthy volunteers, ethics committees always address specific questions on the overall merit of the research project to ensure that the study design is appropriate and the scientific objectives are deemed worthwhile and to ensure the volunteer's radiation exposure is minimized (Huda and Scrimger, 1989). This study has provided evidence on the scientific basic of the labelling technique by validating the neutron activation protocols for the studied formulation to produce the desired radioactivity for *in vivo* research studies. It is a standard and crucial procedure to verify the radioactivity from each radioactive tracer prior to administration to a human.

One of the limitations with neutron activation is the activation of radioactivity from trace elements such as Na-24 within the formulation. Gamma spectroscopy was used to identify the primary radioactivity (Sm-153) and the radioactive by-products within the activated formulation. Previous studies (Honkanen et al., 2004; Marvola et al., 2004) used the acceptance limits for the radioactivity such that any peak area not originating from Sm-153 should not be greater than 0.3% of the Sm-153 main peak area at 103 keV, and that the total for the net peak areas not originating from Sm-153 should not be greater than 1% of the Sm-153 main peak area at 103 keV. In this study, only Na-24 (1368.6 keV) was detected other than the main radionuclide Sm-153 (103 keV). The total net peak area originating from Na-24 was at 0.3% of the Sm-153 main peak area, which was within the acceptance limits. However, in order to minimize radiation burden to volunteer subjects, the formulations were stored for at least 48 h to allow decay of any radioactive by-products, i.e. Na-24.

Although the use of enriched samarium has previously been recommended due to the lower amounts of samarium used and shorter irradiation times (Awang et al., 1993), we have found the use of natural samarium oxide to be acceptable. One further consideration on the activation of natural samarium oxide is the possible production of radioactive Sm-151 which has a physical half-life of 93 years and decays by emitting 20 keV beta radiation. In the present study, an estimation of activity of about 20 Bq from Sm-151 was produced in each tablet. According to the Human Health Fact Sheet published by Department of Energy, United State in 2005, the radiological risk coefficient for Sm-151 is interpreted as lifetime cancer mortality risks, which are 8.6×10^{-12} per pCi intake by inhalation, and 4.6×10^{-13} per pCi intake by ingestion. Accordingly, the lifetime cancer mortality risk from the ingested Sm-151 of activity of 20 Bq is 1.6×10^{-10}, or 1.6 in a 10 billion population. The potential radiation effect arises from Sm-151 is therefore considered negligible in this study.

The neutron activation protocol used in the present study was applied to achieve radioactivity of 1 MBq of Sm-153 at 48 h after irradiation. From the radioactivity assays performed, the protocol was validated to consistently produce the radioactivity at the desired range with an acceptance limit of $\pm 10\%$ from 1 MBq at 48 h following neutron activation. On each study occasions, the administration of tablets to the volunteer subjects was planned at 59 h after neutron activation. Result shows that the mean radioactivity administered to the volunteer subjects was 0.86 ± 0.03 MBq. This corresponded to 0.7 mSv effective dose confirming that the radiation dose was under the dose reference level which was 0.8 mSv as specified in the ethical study approval. Fig. 1 demonstrates the reproducibility of radioactivity over a prolonged period of 24 weeks. Overall the amounts of radioactivity produced were consistent and although a cyclical pattern of the activity contained in the tablets could be seen over time, no clear trend effect was found. Since for each irradiation, six tablets were packed into one ampoule it was expected that the radioactivity in each tablet would vary due to geometrical factors. This needs to be considered together with any spatial variations in the reactor neutron flux and the amount of samarium oxide loaded into each tablet when accounting for the range of activities produced over the entire length of the study.

In conclusion, this study has assessed the reproducibility of Sm-153 activation in a well characterized batch of enteric-coated sustained release guaifenesin 83 mg core tablets over a period of 24 weeks. This has also confirmed that the radioactivity administered did not exceed the dose reference level of 0.8 mSv set by the ethical and UK regulatory approvals for human administration.

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