# Development of Novel F-DOPA Radiopharmaceutical Formulation in Neurodegenerative Disorders and Cancer

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Here we investigated on novel radioactive tracers based on 18F-DOPA helpful for the early diagnosis of the degenerative diseases. 18F-DOPA is available on the market (IASOdopa®) and is indicated in the evaluation of the dopaminergic functions in the striatum and for diagnosis of cancers. The use of these formulations is limited by instability and by injection site reactions such as pain. The aim of this project is to identify new FDOPA formulations stable and better tolerated than currently available. In vitro and in vivo experiments were conducted in comparison with the simulated IASODOPA (sIASO).

In vitro experiments were performed for four new formulations made by the groups of Pharm Technology (Dept Pharmacy-Drug Science, Univ of Bari) on skeletal muscle fibers, renal tsA201 and neuronal SHSY5H cell lines. The range of concentrations tested was 0.005-0.5 mg/mL. The cell viability was evaluated by using the Scepter™2.0 cell counter (MERK-Millipore, USA). Two formulations among those under study were identified. The non cytotoxic concentrations were also identified. The first selected formulation was stabilized using lactic acid (F1) and the second one using cyclodextrins (F2). The applied in vivo procedure was a Single Dose Toxicity Study in which the acute toxicity was evaluated after a single e.v dose in rats and mice within 48 h from the administrations and after a follow up period of 14 days in metabolic cages for the evaluation of not reversible effects. The animals were sacrificed and all the organs and blood collected for further analysis at the end of the follow up (Verbruggen et al., 2008; EMA/CHMP/CVMP/JEG-3Rs/169839/2011-Rev.1). I.m, i.p and s.c palmar tolerability tests were also performed. The highest concentration tested was: 10 mg/mL for F1 and sIASO, 5 mg/mL for F2 formulations. Aliquots (0.2 ml) of these solutions were injected to rats to give a corresponding mass dose of 2 mg in a man of 70 kg of body weight.

Tolerability tests in rats at high doses showed no significant effects on i.p. and s.c. injection on pain for all the formulations under investigations. However, we observed immediately after i.m administration of sIASO 10 mg/mL redness and darkening of the tibialis muscle which was reversible within 2 hours. This phenomenon did not occur with F1 and F2 formulations.

The single dose e.v. study in rats showed a significant drop in body weight between experimental groups (ANOVA one way, $F=4.15$; $p<0.004$) and Bonferroni test showed a significant difference between the group receiving sIASO (10mg/mL) and control group (t-student $p< 0.0039$). This phenomenon was not explained by changes in organ weights and/or the metabolic and physiological parameters of animals. The analysis carried out on plasma samples collected after e.v administration in rats showed a variability among experimental groups regarding the CK (ANOVA one way, $F=5.505$; $p=0.0007$) and the Bonferroni test showed a difference between the group that received the vehicle F2 and other experimental groups ($p<0.005$), while other biochemical parameters were not affected.

Gene expression studies showed a significant increase of genes involved in necrosis, oxidative stress, inflammation and pain, in the brain of the animals receiving sIASO i.m and e.v but not for the other F1 and F2. In parallel with the increase of Murf-1 and atrogin-1 genes the histological analysis on rat tibialis muscles showed a greater density of necrotic and atrophic fibers in the samples from rats i.m treated with vehicle sIASO and sIASO.

Confirmatory experiments in mice demonstrated a significant loss of body weight even in this specie treated with sIASO 10 mg/mL and one fatal event was observed within 6 h following administration.

In conclusion, we found that the F1 formulation based on the lactic acid vehicle was more stable than the other investigated formulations showing a better toxicological profile.

Verbruggen et al. (2008). Eur J Nucl Med Mol Imaging. 35(11), 2144-51.