Intravenous Rutin Nanocrystals with Potential Use for Alzheimer Treatment

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ABSTRACT SUMMARY

Four formulations of rutin anti-oxidant nanocrystals were prepared. To estimate their in vivo behavior, the plasma protein adsorption patterns were analyzed by the 2D-PAGE. The results show that they have similar total amounts of adsorbed proteins, but an important qualitative difference. The Tween 80 coated rutin nanocrystals displayed a high adsorption amount of ApoE indicating the potential for i.v. brain delivery and treatment of Alzheimer disease.

INTRODUCTION

Nanocrystals are a smart formulation to create i.v. injectable formulations of poorly soluble drugs (e.g. NANOEDGE by Baxter, US). In most cases, accumulation in the liver is undesired, the crystals should either circulate in the blood and dissolve, or ideally accumulate in a target region, e.g. the brain. The blood proteins adsorbing on the particles after i.v. injection determine their organ distribution. Analysis of the adsorption patterns allows to estimate the in vivo behavior, and by modification of the particle surface properties the protein pattern can be optimized to reach specify to a certain target (e.g. preferential adsorption of Apo E for brain targeting).

Antioxidants are a major player in potential treatment of Alzheimer disease. Therefore nanocrystals of the poorly soluble anti-oxidant rutin were produced and the protein adsorption patterns were determined to assess their potential for brain targeting after i.v. injection.

EXPERIMENTAL METHODS

Rutin nanocrystals were prepared via high pressure homogenization (Homogenizer Micron LAB 40, APV Deutschland GmbH, Germany) and then stabilized with different surfactants in Milli-Q water (Millipore, Germany). The different nanosuspensions were characterized by photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern Instruments, UK) and laser diffractometry (LD, Mastersizer 2000, Malvern Instruments, UK). The formulations and characterization data of different rutin nanocrystals are shown in Table 1. Compared with the LD results, the increased PCS size of Tween 80-rutin particles may result from a loose bridging effect among the Tween 80 molecules.

The two-dimensional polyacrylamide gel electrophoresis (2D-PAGE; 2-DE) was used to analyze the protein adsorption patterns on particle surface. The particle samples were incubated with human plasma at 37 °C for 5 min (1 ml sample per ml plasma), then centrifugation was used to separate the samples from the excess plasma. The non-adsorbed proteins were washed off and then 2D-PAGE was employed. Proteins were separated on IPG-strips (Serva Electrophoresis, Heidelberg, Germany) according to their isoelectric focusing (IEF) in the first dimension and on gels based on molecular weights (MW) in the second dimension of 2D-PAGE. The spots on gels were recognized by matching the master map of human plasma. The amount of adsorbed protein was analyzed using a semi-quantitative manner based on the spot size and intensity on the gel. Exemplarily, a
2D-PAGE gel of rutin particles is shown in Figure 1.

**RESULTS AND DISCUSSION**

The adsorbed proteins as the biological identities of injected particles control their behaviors *in vivo*. The experimental results show that all the rutin particles adsorbed almost identical total amounts of proteins. For instance, all of rutin particles exhibited almost identically adsorbed amounts on ApoA-IV, ApoJ and ApoA-I, excluding ApoE which is generally regarded as an important protein for targeting to brain [1]. Figure 2 shows in detail the adsorption pattern compositions: the Tween 80 rutin particles (formulation C) possess the adsorbed amount of Apo E at the highest; the rutin particles of A and B have similar adsorbed amounts due to their identical compositions on the surfaces; the Poloxamer 188-rutin particles (D) did not adsorb ApoE perhaps because of the coat with the hydrophilic PEO-chains on the surface [2].

**CONCLUSION**

The four formulations of rutin nanocrystals have similar total amount of adsorbed proteins. Nevertheless, rutin particles coated with Tween 80 on the surface have the highest amount of ApoE. This has the potential to resulting in brain targeting, but their *in vivo* behavior has to be verified in the next step in animals. It opens the perspective of using rutin nanocrystals in Alzheimer treatment.

**REFERENCES**


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