

PŮVODNÍ PRÁCE

Study on ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA renal accumulation using *in vitro* cellular model

ZBYNĚK NOVÝ, JANA MANDÍKOVÁ, FRANTIŠEK TREJTNAR

Charles University in Prague, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Hradec Králové, Czech Republic

Received 30 November 2010 / Accepted 13 December 2010

SUMMARY

Study on ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA renal accumulation using *in vitro* cellular model

Mercaptoacetyltriglycine (MAG3) and dimercaptosuccinic acid (DMSA) labelled with technetium- 99m belongs to standard renal radiodiagnostics. However, the renal transport mechanisms responsible for their high renal uptake have not been fully explained. In addition, no *in vitro* experimental study comparing the renal uptake of these radiopharmaceuticals at the cellular level has not been performed. The investigation compared the ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA renal uptake using primary rat renal cells and evaluated contribution of active and passive transport processes to the renal accumulation. The renal cells were isolated from the rat kidneys by means of the two-phase collagenase perfusion method. The used experimental model showed to be useful tool for such type of investigation. The results documented significant quantitative and qualitative differences in the accumulation of ^{99m}Tc -DMSA and ^{99m}Tc -MAG3 in the rat isolated cells. The found experimental data indicated several times higher uptake of ^{99m}Tc -MAG3 than that found in ^{99m}Tc -DMSA. ^{99m}Tc -MAG3 cellular uptake was substantially decreased when active, energy-dependent processes were inhibited. However, ^{99m}Tc -DMSA accumulation in the renal cells demonstrated only a minor dependency on energy. These findings demonstrate a very different character of the membrane transport determining ^{99m}Tc -DMSA and ^{99m}Tc -MAG3 renal accumulation.

Key words: renal radiodiagnostics – membrane transport – bioelimination**Čes. slov. Farm. 2011; 60, 7–10**

SOUHRN

Studium ledvinné akumulace ^{99m}Tc -MAG3 a ^{99m}Tc -DMSA s využitím *in vitro* buněčného modelu

Merkaptoacetyltriglycin (MAG3) a dimerkaptosukcinát (DMSA), značené techneciem- 99m , patří ke standardním ledvinným radiodiagnostikům. Avšak ledvinné transportní mechanismy, které jsou odpovědné za jejich ledvinnou akumulaci, nejsou dosud plně objasněny. Zatím nebyly provedeny žádné experimentální *in vitro* studie, které by studovaly uptake těchto radiofarmak na buněčné úrovni. Tato práce porovnávala ledvinný uptake ^{99m}Tc -MAG3 a ^{99m}Tc -DMSA s využitím primárních ledvinných buněk potkana a hodnotila účast aktivních a pasivních transportních procesů na této ledvinné akumulaci. Ledvinné buňky byly izolovány z ledvin potkana pomocí dvoustupňové perfúze kolagenázou. Použitý experimentální model se ukázal být vhodným prostředkem pro tento typ výzkumu. Výsledky dokumentují významné kvantitativní a kvalitativní rozdíly v míře akumulace ^{99m}Tc -DMSA a ^{99m}Tc -MAG3 v izolovaných ledvinných buňkách potkana. Získaná experimentální data svědčí o několikanásobně vyšší míře vychytávání ^{99m}Tc -MAG3 v buňkách než

Address for correspondence:

doc. PharmDr. František Trejtnar, CSc.

Charles University in Prague, Faculty of Pharmacy, Department of Pharmacology and Toxicology

Heyrovského 1203, 500 05 Hradec Králové

e-mail: trejtnarf@faf.cuni.cz

v případě ^{99m}Tc -DMSA. Akumulace ^{99m}Tc -MAG3 v buňkách se podstatně snížila při inhibici aktivních, na energii závislých buněčných procesů. U ^{99m}Tc -DMSA ovšem akumulace v ledvinách buňkách vykazovala jen malou závislost ledvinného vychytávání na energii. Tyto nálezy svědčí o velmi rozdílné povaze membránového transportu, který determinuje akumulaci ^{99m}Tc -DMSA a ^{99m}Tc -MAG3 v ledvinách.

Klíčová slova: ledvinná radiodiagnostika – membránový transport – bioeliminace

Čes. slov. Farm. 2011; 60, 7–10

Má

Introduction

Several radiolabelled compounds exerting high cumulation in the kidney have been used in nuclear medicine to imagine the kidneys and to evaluate renal function. To the routinely used renal radiodiagnostics belong mercaptoacetyltriglycine (MAG3) and dimer-captosuccinic acid (DMSA) labelled with technetium- 99m ^{1,2}. Both agents may be accumulated in the kidney by passive or active transport processes. However, the exact renal mechanisms responsible for the high renal accumulation of these radiopharmaceuticals are revealed only partly. In addition, no experimental study comparing the renal uptake of these radiopharmaceuticals at the cellular level has not been performed.

^{99m}Tc -DMSA is well-known renal radiodiagnostics used in nuclear medicine for static imaging of the kidney. ^{99m}Tc -DMSA localizes within the renal tubules and thus can be employed for parenchymal imaging permitting accurate localization of intrarenal masses. Renal scintigraphy indicates the presence of any structural defect in the kidney. Tumors, cysts, abscesses, infarcts, and other space-occupying lesion are seen as spots with decreased radioactivity ³⁻⁵.

^{99m}Tc -MAG3 is a substitute for formerly used [^{131}I]-*o*-iodohippurate (^{131}I -OIH). Compared with ^{99m}Tc -MAG3, ^{131}I -OIH gives poor spatial resolution because the permissible injection dose is limited and its photon energy requires the use of coarse-resolution collimators ⁶. Renal scintigraphy with ^{99m}Tc -MAG3 can provide excellent image quality, even in patients with severely decreased renal function ⁷. ^{99m}Tc -MAG3 is used for both flow and function studies of the kidneys. The most study with ^{99m}Tc -MAG3 is commonly aimed at the measurement of the effective renal plasma flow ⁸.

In this study, an experimental model based on freshly isolated rat renal cells was used to compare the rate of ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA renal uptake and to study general mechanism of the renal accumulation process.

MATERIALS AND METHODS

Chemicals

Mercaptoacetyltriglycine (MAG3) and 2,3-dimer-captosuccinic acid (DMSA) were radiolabelled with

technetium- 99m using commercially available kits (ÚJV, Řež, Czech Republic). The radiochemical purity of both compounds studied was checked by thin layer chromatography according to the procedure given by the producer and was higher than 98%. All the other chemicals used in the experiments were purchased from commercial sources and were of analytical purity grade.

Renal uptake in isolated renal cells

Male Wistar rats weighing 300–380 g were used. The rats were housed under standard conditions (tap water and standard diet, light cycle 12/12 h). Animals were fasting 18–24 h prior to the experiments.

The fresh rat renal cells were isolated from the kidney by means of the two-phase collagenase perfusion method ⁹ according to a previously published modified procedure ¹⁰. Standard incubation of the renal cells with the studied radiolabeled agents was carried out at 37 °C for 30 min. The renal uptake of ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA was determined by mixing 1 ml cell suspension containing $2 \cdot 10^6$ /ml renal cells and 10 μl of the studied compound in saline (radioactivity $\sim 1 \text{ MBq/ml}$). The final concentration of the agents in the incubation mixture was 5 μM . Paralelly, the renal cells were incubated for 30 min under a low incubation temperature (2 °C) to inhibit active transport processes.

After 30 minutes of incubation, 4 ml of ice-cold Krebs-Henseleit buffer was added to the mixture and the cells were separated from the medium by centrifugation (1 min; 120g; 4 °C). Following centrifugation, the supernatant was carefully aspirated and the cells washed again with buffer. Washing of the cells, centrifugation and aspiration of the supernatant were repeated four times under the same conditions. Uptake of the radiolabeled agents in the renal cells was expressed as the percent of radioactivity remaining in the cell fraction.

All the experiments with animals were approved by the Ethical Committee of the Pharmaceutical Faculty, Charles University in Prague, and were carried out in compliance with the respective Czech laws concerning animal protection.

Radioactivity measurements

The ^{99m}Tc -activity in biological samples was measured by a gamma-counter Wallac 1480 Wizard 3 (Wallac, Turku, Finland).

Statistical analysis

Each data point in the figures (and each value in the tables) represents a mean of six experimental values. The found values were compared by the Student t-test. The statistical differences were considered significant at the $P < 0.05$ level.

RESULTS

^{99m}Tc -MAG3 and ^{99m}Tc -DMSA accumulation rate in the isolated rat renal cells found following 30 min incubation at 37 °C are presented in Figure 1. The radioactivity detected in the renal cells was significantly higher in case of ^{99m}Tc -MAG3 in comparison with ^{99m}Tc -DMSA. The performed experiments showed a strong dependency of ^{99m}Tc -MAG3 accumulation rate on incubation conditions since the cellular uptake was many times lower under low temperature (Fig. 2). Similar strong inhibition of the accumulation was not found for ^{99m}Tc -DMSA (Fig. 2). The obtained experimental data indicated in ^{99m}Tc -DMSA only moderately decreased cellular uptake under low temperature.

DISCUSSION

To compare renal uptake of ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA, the freshly isolated rat renal cells were used. This *in vitro* model enables to study transport processes in a less complicated system than in case of *in vivo* conditions. With use of the *in vitro* method, defined conditions and low variability among the individual experiments can be reached. Another advantage of the *in vitro* investigations on the renal uptake of the studied radiodiagnostics is also no interference of the plasma protein binding. Free fraction of the individual agents *in vivo* available for transport into renal cells depends on drug plasma protein binding. As a result, the real concentration useful for the transport may be incomparable. The situation *in vitro* is different since the concentration of tested compounds can be well defined and they are not influenced by the protein binding.

The used viability test based on the trypan blue exclusion showed in our experiments 87–93% of viable renal cells in the obtained cell suspension. The relative high MAG3 accumulation confirmed the high viability of the cellular preparation used to the investigation. The found high rate of ^{99m}Tc -MAG3 uptake in the rat renal isolated cells documents well preserved functions of the transport membrane systems. Therefore, the used experimental model may be potentially useful in the future for *in vitro* studies on the mechanisms of the renal transport of radiolabelled compounds including renal diagnostics agents.

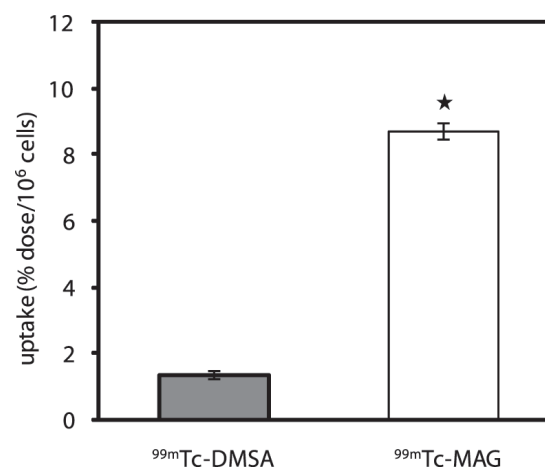


Fig. 1. Uptake of ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA in the isolated rat renal cells following 30 min incubation at 37 °C (mean \pm S.D.) (* = significant difference at $P < 0.05$)

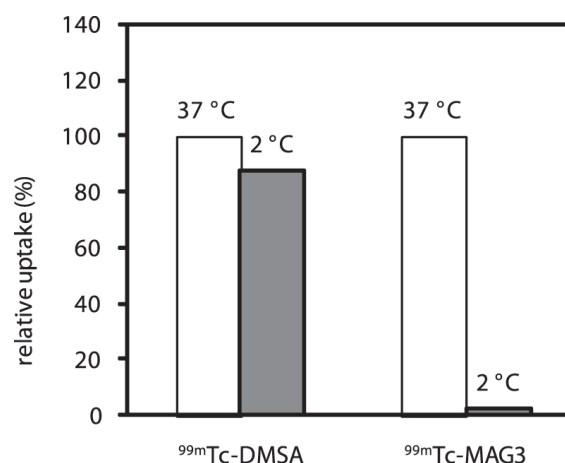


Fig. 2. Relative uptake of ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA in the rat renal cells under normal (100%) or lower incubation temperature

The found experimental data obtained with use of isolated rat renal cells showed different rate of the cellular uptake in ^{99m}Tc -MAG3 and ^{99m}Tc -DMSA *in vitro*. The accumulation of ^{99m}Tc -MAG3 was more than six times higher than that of ^{99m}Tc -DMSA. ^{99m}Tc -MAG3 cellular uptake was substantially decreased under low incubation temperature when energy-dependent processes including the active transmembrane transport are inhibited. However, the same experiment in ^{99m}Tc -DMSA did not demonstrated so intensive dependency of the renal uptake on energy. In conclusion, the experimental data demonstrated substantial differences in the renal transport mechanisms of the compounds under study.

A specific renal transport system exists for anionic compounds^{11, 12)} which seems to be responsible for the secretion of ^{99m}Tc -MAG3 *in vivo*^{13, 14)}. The organic acid transporter (OAT) is located at the basolateral membrane of the renal proximal tubular cells and mediates the uptake of organic acids from the blood *in vivo*^{11, 12)}. Using OAT1-expressing *Xenopus laevis* oocytes, organic anion transporter 1 has been shown to contribute to

^{99m}Tc -MAG3 influx. This active transport can be inhibited by *p*-aminohippuric acid, *o*-I-hippurate, probenecid, glucoheptonate or by other compounds having affinity to the transporter ¹⁴. OAT may be also the responsible influx transporter mediating the renal accumulation in the rat renal isolated cells. However, an open problem remains to be the question whether organic anion transporter 1 is exclusive influx mechanism or other transporters may contribute to the renal transport into renal cells.

Despite its long-term and frequent clinical use, the mechanisms of ^{99m}Tc -DMSA accumulation in the renal tissue is still the subject of debate. Two major possibilities how the agent may enter renal cells have been proposed: tubular reabsorption of the filtered ^{99m}Tc -DMSA from the luminal side or direct uptake from the adjacent peritubular capillaries ^{15, 16}. We demonstrated only relative low contribution of active transport processes to ^{99m}Tc -DMSA accumulation in the isolated rat renal cells. This finding could indicate a relatively low significance of active transport for the retention of ^{99m}Tc -DMSA in the renal tissue. Regardless the direction of ^{99m}Tc -DMSA enter into renal cells in the kidney tissue, a dominant role of passive transmembrane transport mechanisms seems to be probable.

This work was supported by grant No. 124409/FaF/C-LEK of the Grant Agency of Charles University

REFERENCES

1. **Piepsz, A.:** Radionuclide studies in paediatric nephro-urology. *Eur. J. Radiol.* 2002; 43: 146–153.
2. **Peters, A. M.:** Scintigraphic imaging of renal function. *Exp. Nephrol.* 1998; 6: 391–397.
3. **Handmaker, H., Young, B. W., Lowenstein, J. M.:** Clinical experience with ^{99m}Tc -DMSA (dimercapto-succinic acid., a new renal-imaging agent. *J. Nucl. Med.* 1975; 16: 28–32.
4. **Brenner, M., Bonta, D., Eslamy, H., Ziessman, H. A.:** Comparison of ^{99m}Tc -DMSA dual-head SPECT versus high-resolution parallel-hole planar imaging for the detection of renal cortical defects. *Am. J. Roentgenol.* 2009; 193: 333–337.
5. **Taylor, A. Jr.:** Quantitation of renal function with static imaging agents. *Semin. Nucl. Med.* 1982; 12: 330–344.
6. **Taylor, A., Eshima, D., Fritzberg, A. R., Christian, P. E., Kasina, S.:** Comparison of iodine-131 OIH and technetium-99m MAG3 renal imaging in volunteers. *J. Nucl. Med.* 1986; 27: 795–803.
7. **Itoh, K.:** ^{99m}Tc -MAG3: review of pharmacokinetics, clinical application to renal diseases and quantification of renal function. *Ann. Nucl. Med.* 2001; 15: 179–190.
8. **Taylor, A., Eshima, D., Alazraki, N.:** ^{99m}Tc -MAG3 – a new renal imaging agent: Preliminary results in patients. *Eur. J. Nucl. Med.* 1987; 12: 510–514.
9. **Jones D. P., Sundby G. B., Ormstad K., Orrenius S.:** Use of isolated kidney cells for study of drug metabolism. *Biochem. Pharmacol.* 1979; 28: 929–935.
10. **Trejtner, F., Nový, Z., Petřík, M., Lázníčková, A., Melicharová, L., Vaňková, M., Lázníček, M.:** In vitro comparison of renal handling and uptake of two somatostatin receptor-specific peptides labeled with indium-111. *Ann. Nucl. Med.* 2008; 22: 859–867.
11. **Van Aubel, R. A. M. H., Masereeuw, R., Russel, F. G. M.:** Molecular pharmacology of renal anion transporters. *Am. J. Renal Physiol.* 2000; 279: F216–F232.
12. **VanWert, A. L., Gionfriddo, M. R., Sweet, D. H.:** Organic anion transporters: discovery, pharmacology, regulation and roles in pathophysiology. *Biopharm. Drug Dispos.* 2010; 31: 1–71.
13. **Blaufox M. D.:** Transport of ^{99m}Tc -MAG3 via rat renal organic anion. *J. Nucl. Med.* 2004; 45: 86–88.
14. **Shikano, N., Kanai, Y., Kawai, K., Ishikawa, N., Endou, H.:** Transport of ^{99m}Tc -MAG3 via rat renal organic anion transporter 1. *J. Nucl. Med.* 2004; 45: 80–85.
15. **Müller-Suur, R., Gutsche, H. U.:** Tubular reabsorption of technetium-99m-DMSA. *J. Nucl. Med.* 1995; 36: 1654–1658.
16. **de Lange, M. J., Piers, D. A., Kosterink J. G., van Luijk W. H., Meijer S., de Zeeuw D., van der Hem G. K.:** Renal handling of technetium-99m DMSA: evidence for glomerular filtration and peritubular uptake. *J. Nucl. Med.* 1989; 30: 1219–23.