ATTEMPT OF DRUG TARGETING USING MODEL CYTOSTATIC AGENT AND EXCIPIENT (EXPERIMENTAL RABBITS)

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Summary

Selective biodistribution (targeted to liver only, without adverse reactions in other organs) of a model cytostatic agent, ^{131}I-radiolabelled docetaxel, was studied with lipiodol (20% emulsion of lipophilic x-ray contrast, ethiodized poppy seed oil) as an excipient. ^{131}I-lipiodol alone was compared with a mixture of ^{131}I-docetaxel + non-radiolabelled lipiodol. Emulsions were administered to rabbits in ketamine-xylasine anaesthesia by slow infusion either to v. portae (after laparotomy) or to v. jugularis. Time-dependence of biodistribution was qualitatively estimated by 12-min whole body scintigraphy with gammacamera, whereas samples of blood and organs (taken in the pharmacokinetic „steady-state"), measured by gammacounter, yielded quantitative data of radioactivity in individual body compartments. Preferential accumulation was observed for both emulsions: ^{131}I-lipiodol alone, intraportally, showed initial radioactivity localization in the liver and a slightly delayed pulmonary distribution, whereas the ^{131}I-docetaxel-lipiodol mixture, after the initial capture in liver and lungs, showed some radioactivity spreading to other body areas incl. head and bile. For intrajugulary administration of lipiodol alone, localization was observed in the order: pulmonary area, upper abdomen (liver, spleen); for the ^{131}I-docetaxel-lipiodol mixture, capture proceeds through lungs and abdomen (liver, spleen, kidneys), too, spreading further to head and other organs.

Introduction

The study is based upon the idea of the earlier described target biodistributions of some anticancer drugs. An attempt has been performed of a selective ("target - to liver only") biodistribution of the cytostatic agent docetaxel by means of lipiodol as an excipient. The aim was to reduce adverse effects and reactions of a cytostatic in other organs.

The treatment of hepatocellular carcinoma remains poor, and new treatment strategies are needed. Lipiodol, a stable fatty acid ethylester derived from poppy-seed oil, contains 38 % of iodine (by weight) and is retained by hepatocellular carcinoma after intra-arterial injection more then 3 months and by metastatic liver cancer more then 3 weeks (1–3). Intra-arterial ^{131}I-lipiodol has been used to treat inoperable hepatocellular carcinoma and is well tolerated and effective in small tumours (4). Nagamitsu et al. used mitomycin C dissolved in lipiodol for targeted chemotherapy for VX2 tumour implanted in the colon by rabbits (3). Similarly Zhang et al. (6) and Han et al. (7) performed targeted chemotherapy with adriamycine or doxorubicine dissolved in lipiodol. We examined docetaxel labelled with ^{131}I dissolved in lipiodol and ^{131}I-lipiodol for selective biodistribution using scintigraphy as a detection method.

Methodological principle

A comparison of the biodistribution of radioactively labelled (^{131}I) docetaxel (emulsified in lipiodol) and the biodistribution of radioactively labelled (^{131}I) lipiodol on administration:

- into the portal vein (primary biodistribution into the liver),
- into the jugular vein (biodistribution into the whole systemic circulation).

Methods

A. Substances used:

a) ^{131}I-docetaxel: radioactive labelling of the commercial substance (Taxotere®, Rhone-Poulenc, Rorer Canada) was carried out by the Institute of Nuclear Research, Řež, Czech Republic

b) lipiodol as the excipient of docetaxel (LIPIODOL--ULTRAFLUID®, BYK Gulden Lomberg GmbH, Konstanz, Germany)
c) ^{131}I-lipiodol (Lipiocis®, manufactured by CIS Bio International, France).

All substances were administered in the form of a 20% emulsion with the use of the emulsifier Poloxamer 188 + Polysorbate 20.

B. Experimental animals:

Rabbits, Chinchilla, males, weight: 3.65 ± 0.35 kg.

During the experiment (duration about 15 minutes) total anaesthesia induced by i.m. administration of ketamine + xylazine.

C. Procedure:

Groups of rabbits (n = 3 in each group):

a) Group "LP": after laparotomy, 1 ml of a 20% emulsion of ^{131}I-lipiodol (activity = 20 MBq) administered into the portal vein (60 s) by infusion
b) Group "LJ": ^{131}I-lipiodol in the form of an emulsion (in the same way as in a/) administered into the exposed jugular vein
c) Group "DP": ^{131}I-docetaxel emulsified in radioactively non-labelled lipiodol (activity = 20 MBq) administered into the portal vein (in the same way as in a/)
d) Group "DJ": ^{131}I-docetaxel administered into the jugular vein (in the same way as in c/)

D. Parameters under evaluation:

1) Qualitatively: scintigraphy recorded by a gamma-camera (apparatus: MB 9200, Gamma--Müvek, Hungary):
   - a dynamic scintigraphy for a period of 5 minutes from the beginning of the administration of tested emulsions,
   - a static picture for an interval of 5–12 min from the beginning of administration.
2) Quantitatively: in the supposed biodistributional steady-state stage (i.e. after examination by a gamma-camera) animals were killed (i.v. overdose of an anaesthetic mixture and exsanguination) and samples of the blood, urine, bile, and individual organs were withdrawn for radioactivity determination using a gamma-counter (apparatus 1480 Wizard 3, Wallac, Turku, Finland).

Results

1) Examination using a gamma-camera:
   a) Group LP: the primary biodistributional capture of radioactivity after administration of ^{131}I-lipiodol (as the selected excipient) into the portal vein is in the abdominal region (probably the liver and the spleen) see Fig. 1, at further time intervals (after approximately 2 minutes) also in the thoracic region.
   b) Group LJ: the primary capture of radioactivity after ^{131}I-lipiodol administration into the jugular vein is in the thoracic region (Fig. 2), at further intervals also in the abdominal region.
   c) Group DP: after administration of ^{131}I-docetaxel emulsified in lipiodol into the portal vein, the primary capture is in the liver region (Fig. 3), immediately afterwards (after about 2 min) radioactivity is "transported" into the thoracic region and into other regions of the body (including the head). After 5 minutes radioactivity is "not limited to regions".
   d) Group DJ: after administration of ^{131}I-docetaxel into the jugular vein, the highest radioactivity is first in the thoracic region (Fig. 4), immediately afterwards also in the abdominal and cephalic regions, and until the 5th minute, radioactivity is not limited to individual organs.
2) Examination using a gamma-counter:

Quantification of radioactivity in the steady-state biodistributional stage has shown:

   a) Group LP (Fig. 5): after administration of ^{131}I-lipiodol into the portal vein, radioactivity is high in the liver, lungs, and the spleen, in other biological samples it is very low.
   b) Group LJ (Fig. 6): after administration of ^{131}I-lipiodol into the jugular vein, the highest radioactivity is in the spleen and lungs, it is lower by an order in the liver, in other biological samples it is very low.
   c) Group DP (Fig. 5): after administration of ^{131}I-docetaxel into the portal vein, the intensity of radioactivity is of an identical order in the blood, liver, kidney, lungs, spleen, heart, and bile (in spite of a short interval between administration and detection).
   d) Group DJ (Fig. 6): after administration of ^{131}I-docetaxel into the jugular vein, biodistribution of radioactivity is practically similar to that of group DP.

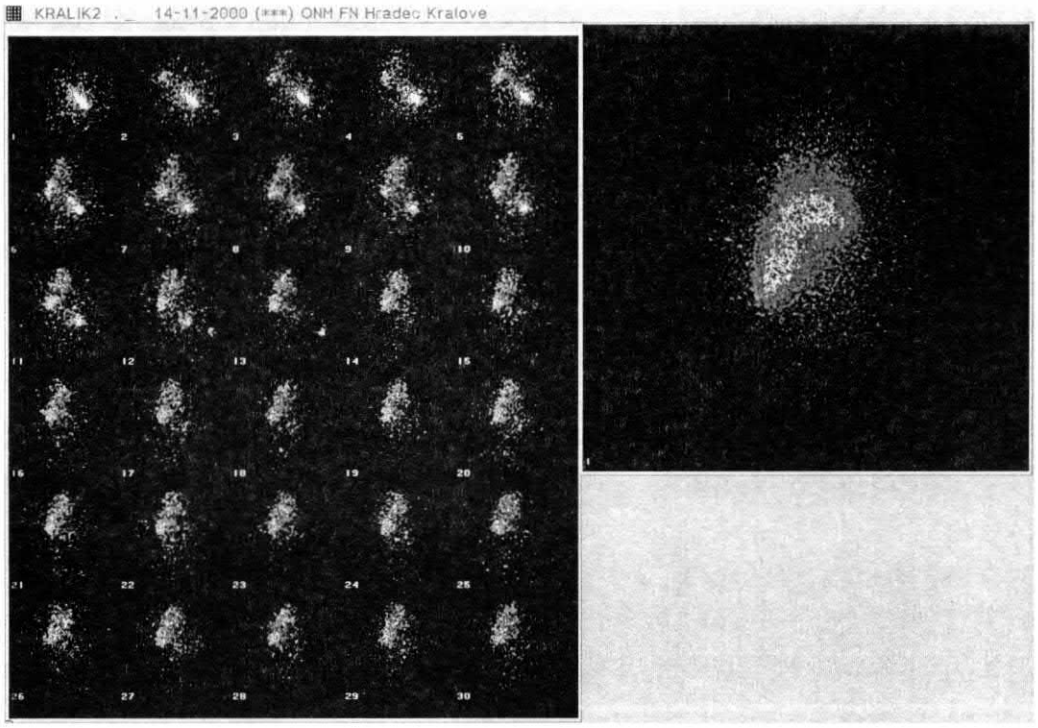

Fig. 1: ^{131}I-lipiodol administered into the portal vein (dynamic scintigraphy 0–5 min. pictures no. 1–30, static mode 5.–12. min - see right big picture)

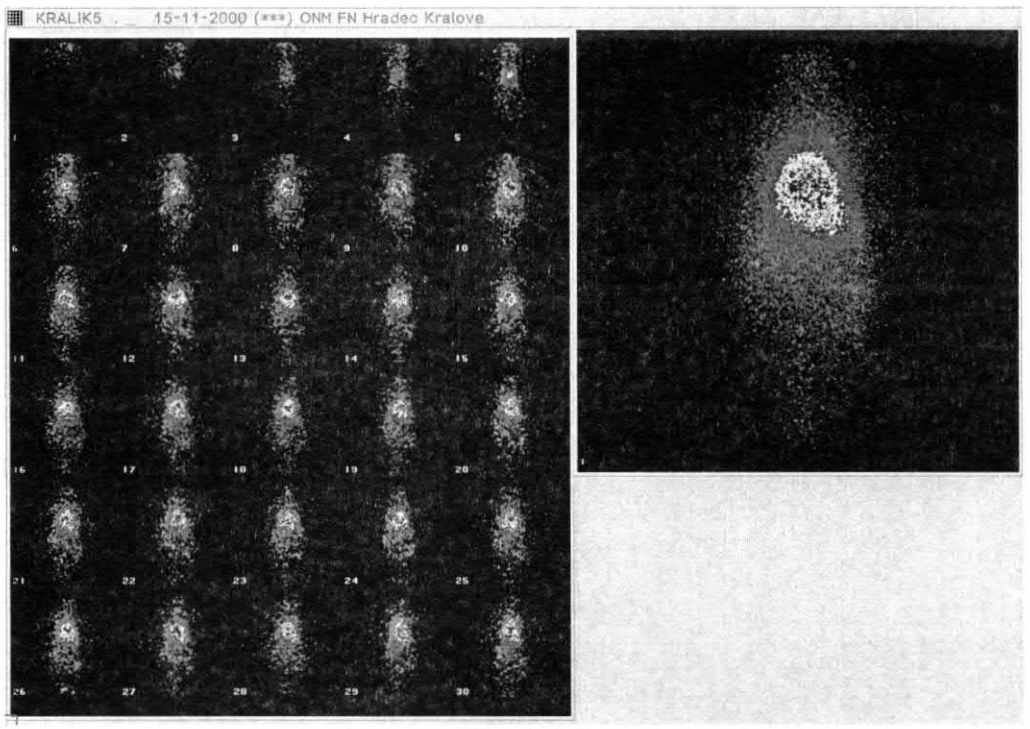

Fig. 2: ^{131}I-lipiodol administered into the jugular vein (dynamic scintigraphy 0–5 min. pictures no. 1–30, static mode 5.–12. min - see right big picture)

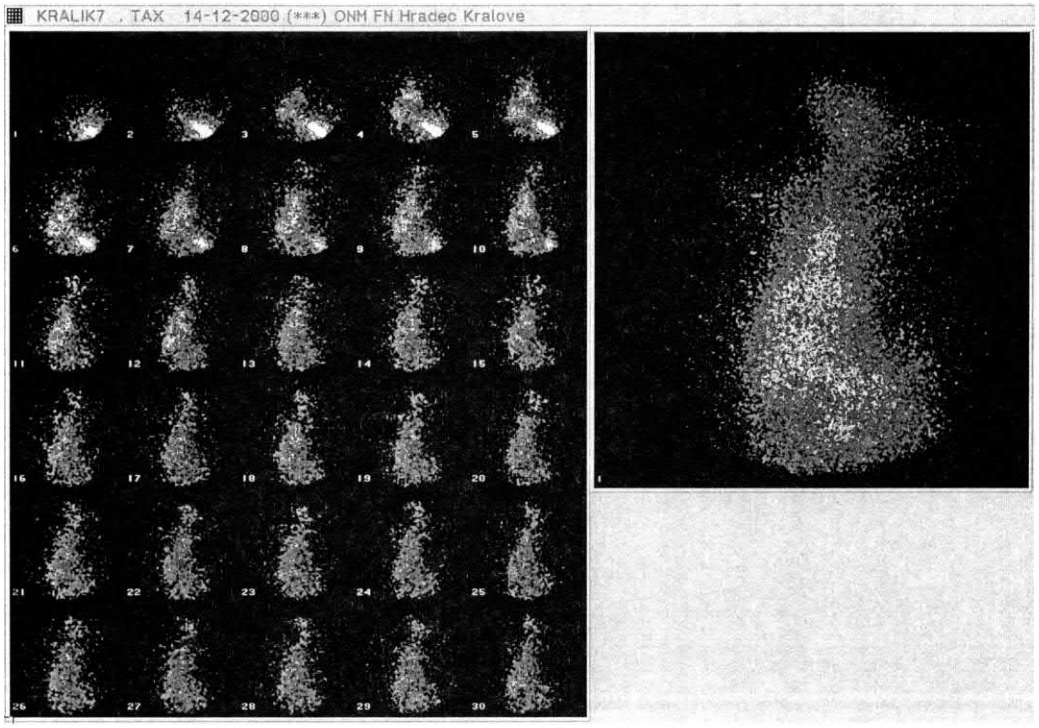

Fig. 3: ^{131}I-docetaxel administered into the portal vein (dynamic scintigraphy 0–5 min. pictures no. 1-30, static mode 5.–12. min - see right big picture)

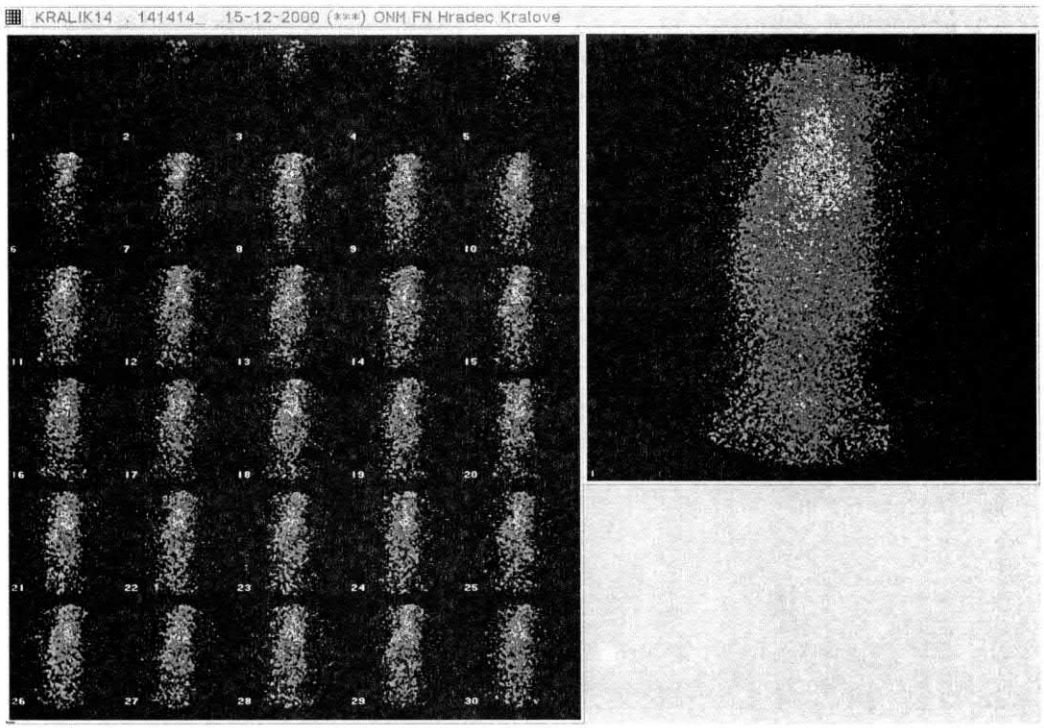

Fig. 4: ^{131}I-docetaxel administered into the jugular vein (dynamic scintigraphy 0–5 min. pictures no. 1–30, static mode 5.–12. min - see right big picture)

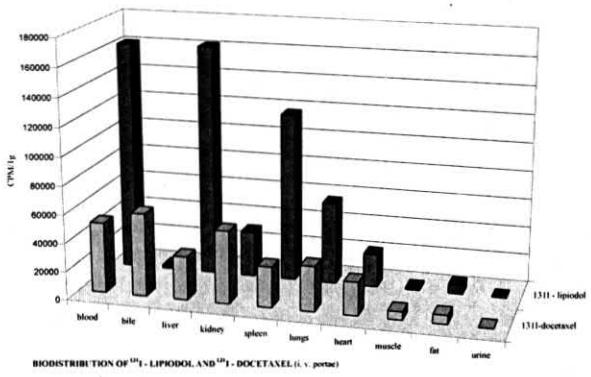

Fig. 5: ^{131}I-lipiodol and ^{131}I-docetaxel administered into the portal vein: biodistribution of radioactivity in the steady-state stage.

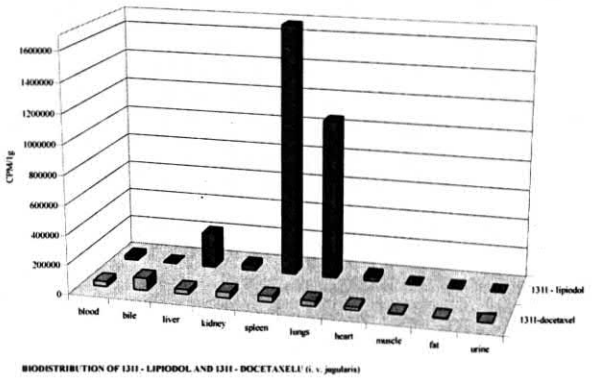

Fig. 6: ^{131}I-lipiodol and ^{131}I-docetaxel administered into the jugular vein: biodistribution of radioactivity in the steady-state stage.

Conclusions and discussion

Combined investigation between the dynamics (time shifts) of biodistribution of radioactivity and quantitative determination of radioactivity in the individual compartments of the organism (in the steady-state biodistributional stage):

- has confirmed the assumption that the primary capture of ^{131}I-lipiodol (the selected excipient) administered in the form of emulsion:
  - into the portal vein takes place in the liver and subsequently in the lungs
  - into the jugular vein (direct into the systemic circulation) takes place in the spleen, lungs, and liver,
- has not demonstrated an agreement between the biodistribution of ^{131}I-lipiodol and the biodistribution of ^{131}I-docetaxel (emulsified in lipiodol): ^{131}I-docetaxel is practically uniformly distributed into all organs both after administration into the jugular vein and after administration into the portal vein.

The former thesis, that docetaxel emulsified in lipiodol will have the same kinetic biodistribution like lipiodol, was not confirmed. Although docetaxel is lipophylic agent, after i.v. application of ^{131}I-docetaxel-lipiodol emulsion quick separation of ^{131}I-docetaxel from lipiodol is occured.

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