Functional recovery after liver resection
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The application of $^{99m}$Tc-GSA scintigraphy with SPECT for the assessment of hepatic function and functional volume during liver regeneration in a rat model
Introduction

Despite advances made in the field of medical oncology, such as radiofrequency ablation (RFA) and arterial chemo-embolization (TACE), partial liver resection remains the most effective treatment for primary and secondary liver malignancies. To ensure safe postoperative recovery, sufficient remnant liver volume and function is crucial as posthepatectomy liver failure still has high mortality. Preoperative volumetric assessment of the future remnant (FRL) liver is widely used as a guideline to exclude patients from liver resection or to select patients who will benefit from preoperative interventions to increase the FRL, such as portal vein embolization (PVE). However, hepatic volume does not always correlate with hepatocellular function, especially in livers with parenchymal disease, such as steatosis, fibrosis and cirrhosis.

Hepatobiliary scintigraphy (HBS) with technetium-99m (99mTc) labeled iminodiacetic acid (IDA) analogues has been introduced to enable accurate quantitative evaluation of total and regional hepatocellular function in patients considered for liver resection. The hepatic uptake of IDA-analogues is similar to the uptake of organic anions such as bilirubin. It has recently been shown that preoperatively calculated remnant liver function (RLF) using HBS correlates with the actual postoperative liver function. Furthermore, HBS has the potential for predicting postoperative complications.

In the clinical situation, HBS, both as planar dynamic scintigraphy and Single Photon Emission Computed Tomography (SPECT), is performed for the assessment of total and regional liver function. Furthermore, the concept of functional liver volume as predictor for posthepatectomy liver failure is currently under investigation. Therefore, HBS is combined with a modern fast SPECT-CT acquisition for the assessment of total and segmental liver function as well as liver functional volume.

For advancement in liver surgery, small animal models are crucial in order to gain more insights in the complex recovery mechanisms of hepatocellular volume and function that occur during liver regeneration. To facilitate and reduce the number of animals needed for research in this field, non-invasive assessment of liver function has been introduced using 99mTc-mebrofenin HBS in both acute and chronic experimental animal models. However, scintigraphic imaging acquisition is restricted to planar modalities since hepatic IDA-kinetics in small animals are too fast for (pinhole) SPECT acquisition.

An alternative radiopharmaceutical for liver function scintigraphy is 99mTc-DTPA-galactosyl serum albumin (99mTc-GSA). Although it is not registered for clinical use outside Japan, 99mTc-GSA is available for pre-clinical investigation elsewhere. 99mTc-GSA scintigraphy is based on the hepatic pharmacokinetics of asialoglycoproteins (ASGP). ASGP is a serum galactose-terminated glycoprotein, which binds to the ASGP receptor (ASGPR) and is subsequently internalized via receptor mediated endocytosis. The ASGPR is expressed in large quantities on the hepatocyte sinusoidal surface. The expression of ASGPR as well as serum accumulation of asialoglycoproteins reflects the severity of chronic liver disease such as hepatocellular carcinoma and cirrhosis in patients. 99mTc-GSA was developed as a synthetic asialoglycoprotein to visualize and quantify its hepatic binding to the ASGP receptor. After intravenous injection and hepatic uptake, 99mTc-GSA remains trapped in the liver for at least 30 minutes. This would potentially enable pinhole SPECT acquisition...
in small laboratory animals for the assessment of both liver function and liver functional volume.

The aim of this study was to evaluate the use of $^{99m}$Tc-GSA scintigraphy as a liver function imaging technique in normal and regenerating rat liver and to validate the use of $^{99m}$Tc-GSA SPECT for additional liver functional volume assessment in a surgical rat model.

**Materials and methods**

**Animals and reagents**

Male Wistar rats (250-300 g, Harlan, Zeist, the Netherlands) were acclimatized for one week under standardized laboratory conditions in a temperature-controlled room with a 12h-light/dark cycle on standard rat chow and water ad libitum. $^{99m}$Tc-GSA was kindly provided by Nihon Mediphysics, Tokyo, Japan. This study was approved by the Animal Ethics and Welfare Committee of the Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

**Camera Design**

For imaging of $^{99m}$Tc-GSA uptake in the rat liver, a gamma camera (Philips ARC 3000, Eindhoven, The Netherlands) available in a dedicated animal care facility was equipped with a pinhole collimator fitted with a 3 mm tungsten insert. The pinhole collimator was facing up. On the detector, a mechanical support was mounted in which the animal is fixed in a Perspex cylinder positioned exactly above the pinhole collimator. The mechanical support was designed in order to place the midline of the cylinder exactly in the middle of the pinhole. The position of the animal is adjustable in the axial dimension. Furthermore, the distance from the cylinder to the pinhole aperture is adjustable. Therefore, this gantry permits optimal pinhole scintigraphy of anterior projections of rats standardizing magnification and orientation. The gamma camera is interfaced to a Hermes acquisition station (Nuclear Diagnostics, Stockholm, Sweden).

**Scintigraphy and Interpretation**

The animals were sedated with a mixture of $O_2/N_2O$ (1:1 V/V, 2 l/min) and isoflurane (1-2 % Florene, Abbott laboratories Ltd, Queensborough, UK). Once anaesthetized, a bolus of 40 MBq $^{99m}$Tc-GSA in 0.3 ml saline was injected in the tail vein. The animals were scanned upon injection of the radiopharmaceutical in anterior position with the liver and the mediastinum in the field-of-view (FOV). For dynamic $^{99m}$Tc-GSA scintigraphy, images were obtained for 30 min (10 min at 5 s per frame and 20 min at 60 s per frame) at the 140 KeV $^{99m}$Tc-peak with a 20 % window in a 128x128 matrix. Data was processed on a Hermes workstation (Nuclear Diagnostics, Sweden). In clinical studies liver function, measured by $^{99m}$Tc-GSA scintigraphy, is expressed by many different, sometimes complex parameters. The most commonly used parameters in planar, dynamic $^{99m}$Tc-GSA scintigraphy are the hepatic uptake ratio of $^{99m}$Tc-GSA (LHL15) and the blood clearance ratio (HH15). We used the $^{99m}$Tc-GSA liver uptake based on a technique described by Ekman et al. The algorithm was adapted for rat hepatobiliary scintigraphy based on the faster hepatic extraction of
Regions of interest (ROI) were drawn around the liver, the heart and large vessels within the mediastinum (serving as blood pool) and around the total FOV (indicative of total activity). The liver ROI was drawn automatically on a threshold-based algorithm using 20% of the maximum liver value on a summed image of the first 2.5 minutes of the acquisition as cut-off. Three different time-activity curves were generated based on the liver, blood pool and total FOV. Liver uptake was calculated in %/min, based on these three parameters. Calculations of hepatic $^{99m}$Tc-GSA uptake were performed using scanned radioactivity values acquired between 60 and 180 seconds post-injection to make sure that calculations were made during a phase of homogenous distribution of the agent in the blood pool and before the rapid phase of hepatic excretion.

The SPECT acquisition was performed 15 min after i.v. injection of $^{99m}$Tc-GSA. Fifty projections (matrix 128x128) were made in a 360° orbit. The acquisition time per projection was 25 seconds. SPECT reconstruction was performed using a Hermes application program adapted to pinhole SPECT, using filtered back projection. A Butterworth post-reconstruction filter (order 5, 0.8 cycles per cm) was applied. After completion of the reconstruction, a threshold for background, 40% of the maximal voxel count value, was applied for determination of the actual liver volume.

Reproducibility

Nine rats were anesthetized by inhalation with a mixture of $O_2/N_2O$ (1:1 V/V, 2 l/min) and isoflurane 1-2% and scanned on two separate days with a two days time interval, to assess the reproducibility of scintigraphic liver function testing and intra-subject variation. Subsequently, a SPECT acquisition of 25 min was made. After the final scintigraphy, livers were removed and the liver volume and weight were determined. Liver volume was calculated as previously described.

Surgical Procedure

Rats were anesthetized by a mixture of $O_2/N_2O$ (1:1 V/V, 2 l/min) and isoflurane 2-2.5% and pain medication was applied by administration of buprenorphine (Temgesic s.c., 0.033 mg/0.1 kg). A midline laparotomy was performed and the liver was mobilized. Partial (70%) heptectomy was performed by resecting median and left lateral lobes. To assess liver regeneration, rats were anaesthetized 1, 3, 5 or 7 days after surgery to perform $^{99m}$Tc-GSA scintigraphy and to determine liver weight and volume, after sacrifice.

Experimental groups

Two groups of rats in different weight categories, group A; 250-300g (n=6) and group B; 300-350g (n=6), were used to compare liver volume and liver functional volume measured by $^{99m}$Tc-GSA SPECT. For the assessment of liver regeneration, 30 rats were randomised into 5 experimental groups. In the control group (n=6) $^{99m}$Tc-GSA scintigraphy was performed to calculate normal $^{99m}$Tc-GSA uptake and liver functional volume. Subsequently, rats were sacrificed to determine liver weight and liver volume. To assess liver regeneration, groups were anaesthetized 1 (n=6), 3 (n=6), 5 (n=6) and 7 (n=6) days after 70% partial heptectomy and $^{99m}$Tc-GSA scintigraphy was performed to calculate $^{99m}$Tc-GSA uptake and liver functional volume. Subsequently, rats were sacrificed to determine liver weight and liver volume.
Statistical Analysis

Commercial computer packages were used for the analysis of the data (GraphPad Prism, SPSS). Values are given as mean ± SEM. The relation between the liver binding of $^{99m}$Tc-GSA and other parameters was tested using the standard Pearson correlation coefficient r. The scintigraphy calculations were further analyzed to the method of Bland-Altman, which is a supplementary method to compare two different methods when the true value is unknown. The data were plotted as scatter plots of the mean values versus the difference of the calculations and evaluated as mean difference and standard deviation of the difference. Differences between postoperative $^{99m}$Tc-GSA uptake and volume measurements were evaluated with a non-parametric repeated measurement Anova Test and Wilcoxon Signed Ranks Test. All statistical tests were two-tailed and differences were evaluated at the 5% level of significance.

Results

Reproducibility of $^{99m}$Tc-GSA calculations

The hepatic uptake of $^{99m}$Tc-GSA was homogenous in all liver lobes (Figure 1). The uptake rate remained constant after 10 min of imaging. The $^{99m}$Tc-GSA uptake was measured twice within the same animal with a time interval of two days. No remaining radioactivity was detected in the second measurement before re-injection. There was a strong correlation between the two measurements (Figure 2A, Pearson $r = 0.75$, $P = 0.019$). The Bland Altman analysis (Figure 2B) shows that all measurement points were located within the 95% confidence interval (from -10.22 to 7.24).

Comparison between functional volume, $^{99m}$Tc-GSA uptake and liver volume in normal livers

Comparison between conventional liver volume and liver functional volume measured by $^{99m}$Tc-GSA SPECT was performed in two groups (n=6) of rats in different weight categories (group A; 250-300 g and group B; 300-350 g). One rat was excluded due to subcutaneous injection of $^{99m}$Tc-GSA. Conventional liver volume and liver functional volume in group A was $12.38 \pm 0.32$ ml and $12.33 \pm 0.25$ ml, respectively, and in group B $18.23 \pm 0.45$ ml and $17.33 \pm 0.68$ ml, respectively. $^{99m}$Tc-GSA uptake in group A was $25.40 \pm 0.87$ %/min and in group B $33.10 \pm 2.07$ %/min. Conventional liver volume, liver functional volume were significantly different between the two groups ($P = 0.0043$, $P = 0.002$ Mann Whitney test). $^{99m}$Tc-GSA uptake was not significantly different between the groups ($P = 0.052$). There was no significant difference between liver volume and functional volume in both groups ($P = 0.079$ and $P = 0.18$). The correlation between liver volume and liver functional volume was strong and significant (Spearman $r = 0.93$, $P < 0.0001$, Figure 3A). The correlation between GSA uptake and liver volume was moderate (Spearman $r = 0.62$, $P = 0.043$, Figure 3B).
Liver volume during liver regeneration

The mean liver volume was decreased one day after 70% PH (6.77 ± 0.12 ml) compared to baseline (12.38 ± 0.32 ml), after which it regenerated from 9.08 ± 0.77 ml at day 3 to normal liver volume at day 5 (12.15 ± 0.45 ml) and day 7 (11.76 ± 0.50 ml, Figure 4A). 99mTc-GSA SPECT was used to calculate the functional liver volume. The mean baseline functional volume was 12.14 ± 0.20 ml. One day after PH, the functional volume was

Figure 1. Dynamic scintigraphy in a control rat after i.v. administration of 40 MBq 99mTc-GSA in the tail vein. The right panel shows refraamed images of the dynamic acquisition. There is homogenous liver uptake from the blood pool activity within 5 min. There is no excretion of tracer within the bile to the intestinal tract. The upper left panel shows a summed image with the liver in the ROI (red line). The lower left panel shows the time-activity curve of the liver ROI, with accumulation to a plateau phase at 10 min post injection.

Figure 2. The correlation between the two measurements of 99mTc-GSA uptake was significant and strong \( r = 0.754, P = 0.019 \) (A). The Bland-Altman analysis shows that all calculations points are located in between the 95% confidence intervals (from -10.22 to 7.24) (B).
reduced to 8.07 ± 0.27 ml. Similar to conventional liver volume, the functional volume increased at day 3 (9.68 ± 0.92 ml) to normal liver functional volume at day 5 (11.82 ± 0.39 ml) and 7 (11.80 ± 0.37 ml) (Figure 4A).

There was a significant difference at day 1 between liver volume and liver functional volume assessed by $^{99m}$Tc-GSA SPECT (6.77 ± 0.12 ml and 8.07 ± 0.27 ml, respectively, $P = 0.048$ Wilcoxon Signed Ranks Test). There were no significant differences between the volumes assessed by both methods at baseline, 3, 5 and 7 days after 70% PH, ($P = 0.14$, $P = 0.71$, $P = 0.69$ and $P = 0.92$, respectively). There was a strong correlation between the two volumes in the regenerating liver (Spearman $r = 0.857$, $P < 0.0001$, Figure 4B).

$^{99m}$Tc-GSA uptake during liver regeneration

The mean baseline level of $^{99m}$Tc-GSA uptake in a normal liver was 27.08 ± 1.82 %/min. One day after 70% PH, the $^{99m}$Tc-GSA uptake (8.01 ± 0.62 %/min) significantly decreased compared to baseline. At day 3, 5 and 7 after resection, $^{99m}$Tc-GSA uptake increased again to 10.34 ± 0.46 %/min, 13.53 ± 1.20 %/min and 21.71 ± 1.94 %/min, respectively (Figure 5).
To investigate if the decrease in uptake of $^{99m}$Tc-GSA was mainly due to the decrease in liver weight after 70% PH, we divided the $^{99m}$Tc-GSA uptake by the liver weight. The mean baseline level of $^{99m}$Tc-GSA uptake per gram liver in a normal liver was $2.44 \pm 0.07$ %/min/g. One day after 70% PH, the $^{99m}$Tc-GSA uptake per gram liver decreased significantly to $1.51 \pm 0.08$ %/min/g (vs baseline, $P < 0.008$, Mann Whitney test) (Table 1). At 3 and 5 days after 70% PH, it decreased to $1.46 \pm 0.13$ %/min/g and $1.32 \pm 0.10$ %/min/g, respectively (vs baseline $P = 0.008$ and $P = 0.004$, respectively, Mann Whitney test). At day 7, the $^{99m}$Tc-GSA uptake per liver weight had returned to baseline level, i.e. $2.13 \pm 0.13$ %/min/g (vs baseline, $P = 0.08$, Mann-Whitney test).

Table 1. $^{99m}$Tc-GSA uptake per liver weight in regenerating liver after 70% PH

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<tr>
<td>$^{99m}$Tc-GSA (%/min/g)</td>
<td>$2.44 \pm 0.17$</td>
<td>$1.51 \pm 0.08$ *</td>
<td>$1.46 \pm 0.13$ *</td>
<td>$1.32 \pm 0.10$ *</td>
<td>$2.18 \pm 0.13$</td>
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* vs baseline, $P < 0.05$, Mann Whitney test

Figure 5. $^{99m}$Tc-GSA uptake decreased the first day after 70% PH, after which it slowly recovered to baseline uptake at day 7 after resection.

Figure 6. During the regeneration process, there was a significant difference between $^{99m}$Tc-GSA uptake and both conventional liver volume ($P < 0.001$), and liver functional volume ($P < 0.001$), when data was expressed as a percentage of baseline levels.

Comparison between $^{99m}$Tc-GSA uptake, liver functional volume and liver volume during liver regeneration

To compare $^{99m}$Tc-GSA uptake, liver functional volume and liver volume during liver regeneration, data was expressed as a percentage of baseline measurement (Figure 6). During the regeneration process, there was no significant difference between liver volume and liver functional volume assessed by $^{99m}$Tc-GSA SPECT ($P = 0.144$, non-parametric repeated measurement Anova). There was a significant difference between $^{99m}$Tc-GSA uptake and liver volume ($P < 0.001$), and liver functional volume ($P < 0.001$). There was a significant difference between $^{99m}$Tc-GSA uptake and liver volume at day 1 ($P < 0.001$), 3 ($P = 0.09$), and 5 ($P < 0.001$) after 70% PH. At day 7 there was no significant difference ($P = 0.14$).
**Discussion**

We report the utility of $^{99m}$Tc-GSA scintigraphy combined with SPECT in a rat model for the assessment of liver function and liver functional volume, both in the normal situation and during liver regeneration.

Liver resection remains the only curative treatment for patients with primary or secondary liver malignancy. Developments in surgical techniques and postoperative care have enabled more extensive and complex liver resections. Extensive resections, however, bear the risk of postoperative complications. The recovery of liver volume is directly related to the size of the resected liver and the regeneration capacity of the remnant liver.

Conventional analysis of liver function, i.e. volumetric evaluation by CT or laboratory tests, does not always correlate with function. This especially concerns patients with underlying parenchymal liver disease, such as steatosis, fibrosis or cirrhosis. Therefore, an accurate preoperative evaluation of FRL function and (functional) volume is crucial for surgeons in planning liver resection. Both $^{99m}$Tc-GSA scintigraphy and HBS, combined with SPECT-CT have the potential to combine both these aspects within one imaging technique.

Currently, the most commonly used radiopharmaceutical in HBS is $^{99m}$Tc-mebrofenin. However, in small animal models the metabolism of $^{99m}$Tc-mebrofenin is too fast (hepatic activity peak approx 2.5 min) to be combined with SPECT for volumetric analysis. This requires a longer scan period (30 min) in rodents because of technical aspects.

$^{99m}$Tc-GSA liver scintigraphy was developed in Japan as a non-invasive liver function test. The liver is the only uptake site for $^{99m}$Tc-GSA and it is therefore an ideal agent for functional liver scintigraphy. After an i.v. bolus of $^{99m}$Tc-GSA, dynamic $^{99m}$Tc-GSA scintigraphy images are obtained by a gamma camera positioned over the heart and liver region. The blood clearance and hepatic uptake are obtained by generating regions of interest (ROIs) from heart and liver. It is assumed that the ligand-receptor binding is a second-order process, in which a significant fraction of receptor is occupied during the course of the imaging study. $^{99m}$Tc-GSA remains trapped in the liver for at least 30 minutes. This enables SPECT acquisitions in small laboratory animals for the assessment of both liver function and liver functional volume.

Clinical studies report a correlation with other liver function tests such as iCG clearance and Child-Turcotte-Pugh classification. However, the broader use of $^{99m}$Tc-GSA liver scintigraphy has been hampered by the fact that it has not been approved for clinical use in Europe and the USA. $^{99m}$Tc-GSA is available for pre-clinical investigation outside of Japan. In the clinical situation, liver function is expressed by many different, sometimes complex parameters. In our study, we applied a method that calculates the hepatic binding rate based on liver $^{99m}$Tc-GSA uptake and the count ratio of liver to blood pool. $^{99m}$Tc-GSA SPECT was used for the calculation of liver functional volume, with the use of the outline extraction method.

In the normal rat liver we demonstrated a strong correlation between conventional liver volume and liver functional volume. During the regeneration process, a significant correlation was seen between conventional liver volume and liver functional volume. Therefore, $^{99m}$Tc-GSA SPECT is an accurate, non-invasive method to assess liver functional volume in normal rat liver.
In patients with a normal liver without underlying liver disease, a strong relation is described between liver volume and liver function. In our study, already a moderate correlation was demonstrated between liver volume and $^{99m}$Tc-GSA uptake in the normal rat liver within data with a small range of values.

After 70% partial hepatectomy, a discrepancy between volumetric ($^{99m}$Tc-GSA SPECT, conventional analysis) and functional recovery ($^{99m}$Tc-GSA uptake) was observed during the regeneration process. $^{99m}$Tc-GSA uptake underestimated the hepatic regeneration in comparison to liver volume. In clinical studies a discrepancy has been described between volumetric regeneration and functional regeneration after partial hepatectomy. Tanaka et al. reported that functional regeneration was impaired in large resections in comparison to the volumetric regeneration. Others concluded that the functional regeneration was more rapid than the morphological regeneration measured by CT-volumetry. However, the data presented in these studies did not provide enough evidence to support their conclusions. Therefore, it is difficult to draw conclusions regarding the difference between functional and volumetric regeneration in the clinical situation. In our study, $^{99m}$Tc-GSA uptake was decreased in the first days after partial liver resection. This decrease was only due to the decrease in liver weight after resection, the $^{99m}$Tc-GSA uptake per gram liver would be comparable with baseline. However, we saw a decrease in $^{99m}$Tc-GSA uptake per gram liver during the first days after resection, which returned to baseline at day 7. The decrease in uptake per gram liver can be explained by a decrease in ASGP receptors per hepatocyte, a decrease in affinity of the ASGP receptor or a decrease in the amount of hepatocytes per gram liver. In vitro studies have described a decrease in surface ASGP receptors during liver regeneration. Mechanism of decreased cell surface ASGP receptors include the secretion of the ASGP receptor into the extracellular space and reduced recycling of the receptor after endocytosis. Our results may support the theory that the amount of ASGP receptors decrease during the early phase of regeneration.

Conclusion

$^{99m}$Tc-GSA scintigraphy combined with SPECT is a feasible, non-invasive method to assess hepatic volume in a normal rat liver, as well as in a regenerating rat liver. However, the hepatic $^{99m}$Tc-GSA uptake underestimates the hepatic regeneration possibly due to a decrease of AGPR in the early phase after partial hepatectomy. Therefore it may not be accurate for the assessment of liver function in a regenerating rat liver.

References


