Chapter 13

The outcomes of hepatic artery embolization in a rabbit liver VX2 tumor model

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Abstract

**Background:** The aim of this study is to determine the effects of trans arterial embolization (TAE) on tumor volume and liver regeneration in a rabbit VX2 tumor model.

**Methods:** Twenty-three rabbits underwent subcapsular tumor implantation with a VX2 tumor.
Two weeks after implantation, eighteen rabbits were used for TAE experiments, five rabbits were used as a sham group. Tumor response and liver regeneration response of the embolized cranial and non-embolized caudal liver lobes were assessed by CT volumetry, liver to body weight index, and the amount of proliferating hepatocytes.

**Results:** All super selective arterial tumor embolization procedures were performed successfully. Despite embolization, the tumor volume increased after an initial steady state. The tumor volume after embolization, was smaller, compared to the sham group, but this difference was not significant. Massive necrosis of the tumor however, was seen after embolization, without damage of the surrounding liver parenchyma. There was a significant atrophy response of the tumor bearing cranial lobe after super-selective arterial embolization of the tumor with a concomitant hypertrophy response of the non-embolized, caudal lobe. This regeneration response was confirmed histologically by a significantly higher number of proliferating hepatocytes on the Ki-67 stained slides.

**Conclusion:** Super selective, bland arterial coil embolization causes massive necrosis of the tumor, despite increase of volume on CT-volumetry. Atrophy of the tumor bearing liver lobe is seen after arterial embolization of the tumor with a concomitant hypertrophy response of the non-embolized lobe, despite absence of histological damage of the tumor-surrounding liver parenchyma.
Introduction

For patients with primary or secondary hepatic tumors, the treatment deemed most effective is liver resection. However, for this intervention to be considered, a minimum volume of remnant liver is required for sufficient liver function and volume after resection. If the future remnant liver (FRL) is too small, patients are considered to be unresectable. Unfortunately, in patients with malignant liver tumors, less than 15 to 20% are eligible for surgical resection.

Portal vein embolization (PVE), in which either the right or left branch of the portal vein is occluded, is an option to increase the volume of FRL by inducing atrophy of the embolized liver lobe, and consequently, hypertrophy of the contralateral, non-occluded liver lobe.[1] However, there is evidence that after PVE the regenerating liver releases growth factors and cytokines, which also enhances tumor growth.[1-8] In addition, PVE results in a compensatory hyperperfusion of the ipsilateral hepatic artery, which will boost the growth of tumors mainly fed by the hepatic artery, such as hepatocellular carcinomas (HCC) and colorectal liver metastases (CRLM).

Embolizing the hepatic arteries supplying the tumor-bearing liver segments is a conceivable treatment option, alone or in combination with PVE. Transcatheter hepatic artery embolization (TAE), with or without additional chemotherapy, may be offered to patients with primary liver tumors or liver metastases who are unsuitable for resection. Embolizing the tumor-supplying artery will decrease arterial perfusion of the tumor, resulting in selective ischemia of the tumor and possibly, reduction of tumor size.

We explored the effects of an arterial embolization strategy as described above in an animal model in which tumor growth rate can be assessed in relation with TAE. The VX2 liver tumor model in rabbits is used, enabling evaluation of liver regeneration response and tumor changes after TAE. The aim of this study was therefore, to determine the effects of TAE on tumor volume and hypertrophy response in the VX2 liver tumor model in rabbits.

Methods

The Institutional Animal Ethics Committee of the Academic Medical Center of the University of Amsterdam approved this study protocol.

Animals

Twenty-three female New Zealand White rabbits (Harlan, Charles River, France) were acclimatized for 2 weeks under standardized laboratory conditions. This included a temperature-controlled room with a 12-hour light/dark cycle and access to standard chow and water ad libitum. The subjects had a mean weight of 3253±288 grams.
Tumor model
After anesthetization of the rabbit, four tumor fragments of 0.5x0.5 mm each were injected superficially in the subcapsular area of the left-medial liver lobe using a 16-gauge angiocatheter. After the angiocatheter was removed, the liver capsule was manually compressed, and the abdomen was closed in two layers. Five rabbits were used as a control group, 18 rabbits were used for the embolization experiments. Two weeks after implantation, the injected VX2 carcinoma had acquired sufficient mass in the rabbit model to be used for the TAE experiments. Two weeks after TAE, and thus four weeks after tumor implantation, the rabbits were sacrificed.

Anaesthesia and TAE conditions
Animals were anesthetized by intramuscular injection of 25.0 mg/kg ketamine (Nimatek, Eurovet, Bladel, the Netherlands) and 0.2 mg/kg dexmedetomidine (Dexdomitor, Orion corporation, Espoo, Finland). The eyes were protected from drying out using an eye cream (Oculentum simplex, Pharmachemie, Haarlem, the Netherlands). After subcutaneous injection of 0.03mg/ kg buprenorphine (Temgesic, Reckitt Benckiser Healthcare Limited, Hull, Great-Britain) and 0.2mg/kg Enrofloxacin (Baytril, Bayer Healthcare, Berlin, Germany) the rabbit was placed in a supine position. Heart rate and arterial oxygen saturation were measured by pulse oximetry (Hewlett Packard M1165A model 56S, Andover, MA) on the hind leg throughout the procedure.

Transcatheter Hepatic Artery Embolization
Hepatic artery embolizations were performed by an interventional radiologist (KPvL) with over 10 years of experience. The left central auricular artery, located in the center of the left ear, was punctured and canulated with an 18-gauge (18G) catheter (Hospira Venisystems, Lake Forest, IL). A 3fr Renegade microcatheter (Boston Scientific, Place Natick, MA) with a 0.014 inch Transend-ex wire (Boston Scientific, Place Natick, MA) was subsequently introduced into the 18G catheter and under fluoroscopic guidance using a mobile C arm (Oldelft Benelux, Veenendaal, The Netherlands), advanced in a retrograde direction through the external carotid artery and the common carotid artery into the aortic arch and the descending aorta. An aortogram was performed, and the origin of the celiac trunk was located. After canulating the celiac trunk and passing the gastric and gastro-duodenal branches, the common hepatic artery was catheterized and the feeding branches of the tumour were visualized. A super-selective embolization was performed using a single 2 mm straight platinum coil (Boston Scientific, Place Natick, MA) as close to the tumor as possible. Finally, control angiography was performed to assure complete embolization of the target vessels, and to verify flow in the spared right cranial and the caudal hepatic arterial branches. After removing the catheter, the puncture site was manually compressed to avoid bleeding.
Angiography

Using a mobile C-arm Exposcop 8000 (Ziehm Imaging, Nürnberg, Germany), angiography was performed at different time points during the experiments. Preceding TAE, the first angiograph is performed to visualize hepatic arterial anatomy and tumor vascularisation. Immediately after TAE a control angiogram is performed to check the result of complete tumor embolization. Finally, on day 14 after TAE the third angiography was executed to confirm complete occlusion of the hepatic artery.

CT volumetry

Tumor growth was determined by means of CT-volumetry. Using a 64-slice CT scan (Brilliance 64-channel, Philips, Eindhoven, The Netherlands), contrast-enhanced multiphasic CT-scans were made for the arterial (15s), portal (30s), and venous phase (45s) after injection of contrast solution (3 mL Visipaque, GE Healthcare, Waukesha, WI) in an ear vein, which was subsequently flushed with 4 mL sterile physiological saline. On each section of the CT scan, total liver, caudal liver lobe and the tumor(s) were outlined manually after which total liver volume (TLV), caudal liver lobe volume (CLV) and tumor volume (TV) were calculated, respectively. CT-scans were made 14 days before embolization (on the day of tumor implantation), directly following TAE, and on day 3, 7, 10 and 14 after TAE. On day 14 after TAE, after the last CT-volumetry, the rabbits were sacrificed. During CT-scans, the rabbits were anesthetized and positioned in supine position.

Liver / tumor volumes

Tumor growth rate (TGR) after TAE was calculated by means of dividing the calculated tumor volume on x days after TAE (dx) by the calculated tumor volume two weeks after tumor implantation (d0), giving us the following formula: TGR = TV_{dx}/TV_{d0}. The volume ratio of the caudal, non-embolized liver lobe to the total liver volume (%CLV) was calculated by dividing the caudal liver lobe volume by the TLV minus the TV, using the following formula: %CLV = CLV/(TLV-TV) x 100%. In a similar way as %CLV, atrophy of the cranial liver lobe (%CRLV) was calculated with the formula: %CRLV = (TLV-CLV-TV)/TLV x 100%.

Liver to body weight index

After sacrifice, the entire liver and the caudal liver lobes were weighed using a precision scale (Sartorius, Göttingen, Germany). Body weight can influence total liver weight; this is why the caudal liver lobe weight was divided by the body weight.

Biochemical assessments

Liver function and liver damage parameters were assessed in blood samples by routine clinical chemistry. These samples were obtained before tumor implantation, after two weeks of tumor growth, and before TAE. Following TAE, blood samples were taken three hours after the procedure, and on post-TAE day 1, 3, 7, 10 and 14. AST (aspartate aminotransferase), ALT (alanine aminotransferase), AP (alkaline phosphatase), γGT
(gamma-glutamyltranspeptidase), and bilirubin were determined to serve as liver damage parameters. Liver synthesis function was determined by measuring plasma prothrombin time and albumin.

**Histological examination**

Biopsies from the embolized liver lobes and the non-embolized liver lobes were taken at sacrifice. Tissue samples were routinely fixed in 4% formalin (48 hours) and then processed to paraffin tissue blocks. 4μm sections of these blocks were cut and stained with haematoxylin and eosin (H&E). The H&E slides were blindly evaluated by an experienced liver pathologist.

Portal inflammation was indiscriminately graded as follows: 0 (absent), 1 (mild), 2 (moderate), or 3 (severe). Sinusoidal dilation was graded as: 0 (absent), 1 (mild; involving ≤ one-third of the (centro-) lobular area), 2 (moderate; involvement ≤ 2/3 of the parenchyma), or 3 (severe; involving ≥ 2/3 of the liver parenchyma). Portal oedema was scored by determining the percentage of portal tracts involved: 0 (not present), 1 (<25%), 2 (25-50%), 3 (50-75%), and 4 (>75%). The presence of areas with merging necrosis of liver parenchyma was scored as: 0 (absent), 1 (affecting <25% of the parenchyma), 2 (affecting 25-50% of the parenchyma), 3 (affecting 50-75% of the parenchyma), and 4 (affecting >75% of the parenchyma).

For the evaluation of hepatocyte proliferation in normal, non-tumorous liver parenchyma of the embolized cranial liver lobes and the non-embolized caudal liver lobe, staining with the proliferation marker Ki67 was performed (monoclonal mouse anti-rat Ki-67 antigen, clone MIB-5, Dako Cytomation, Glostrup, Denmark). The immunostained sections were counterstained with haematoxylin. The immunostained sections were quantified in 10 fields of view per section (40x magnification) using a Leica CMLB microscope (Leica Microsystems, Wetzlar, GmbH, Rijswijk, the Netherlands) and expressed as percentage of the total amount of pixels in the field of view.

**Statistical analysis**

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS 18.0), and GraphPad Prism (GraphPad Software, San Diego, CA). Data are tested for normal distribution, and equal variances. Values are expressed as means ± SEM, unless otherwise stated. Continuous, non-parametric data were compared (TAE versus sham) by the Mann-Whitney U test. The Wilcoxon signed rank test was used for non-parametric continuous data for different time points within groups. Correlation between variables (total/caudal liver volume as measured by CT volumetry and actual liver weight determined at sacrifice) was tested using the Pearson’s r correlation coefficient. Histological specimens were evaluated using the Fisher’s Exact test and Chi-square test where appropriate. Since most of the histology scores are ordinal (there is a ranking in the categories), the linear by linear association test was also used, which is identical to the Fisher’s Exact test. The median of differences between cranial and caudal hepatocyte proliferation, as determined by Ki67
staining, were evaluated using the Wilcoxon signed rank test. Statistical significance was accepted when p<0.05.

Results

Angiography

Selective angiography and subsequent embolization of the hepatic artery using a minimally invasive route, succeeded in all procedures. All initial angiographies, performed before the embolization procedure, showed a normal blood supply by the hepatic artery and an arterial hypervascularization of the tumor in the cranial liver lobes. The control angiography, following super selective embolization, showed no flow in the embolized hepatic artery, and normal flow in the caudal artery and the gastro-duodenal artery. Angiograms performed after 14 days, before sacrifice, again showed normal blood flow in the hepatic artery but no flow in the arterial branches feeding the tumor. The tumor was clearly visible on angiography, as a hypo-vascular mass within the normal enhancing parenchyma of the cranial liver lobe.

Liver regeneration response

Using CT volumetry, absolute total volume measurements were calculated. The absolute data and % CLV rates are presented in table 1 and 2, respectively. The % CLV before TAE, calculated 14 days after tumor implantation, was 22.7±0.7%. The % CLV increased to 24.4±1.1 (p=0.172), 25.3±1.3% (p=0.081), 25.0±0.8% (p=0.032), and 26.6±1.0% (p=0.004) on day 3, 7, 10 and 14, respectively. In the sham-group, there were no significant differences over time. When the time points were analyzed one-by-one comparing both groups, differences were observed from day 7 till 14, post-TAE (table 2 and figure 1A). The volume percentage of the cranial liver lobe significantly decreased from 76.6±0.7% on day 0, to 70.8±1.7% on day 3, 69.9±2.1% on day 7, 68.4±2.1% on day 10, and 64.9±2.5% on day 14 (figure 1B). The total liver volumes as measured by means of CT volumetry correlated well with liver weight at sacrifice (r=0.965, p<0.001) (figure 2A). This was also the case for the caudal liver lobe calculated by CT volumetry, which correlated strongly with the actual weight of the caudal liver lobe at sacrifice (r=0.905, p<0.001) (figure 2B).

Table 1. Absolute measurements of CT volumetry data of total, caudal, cranial and tumor volumes before and after TAE.

<table>
<thead>
<tr>
<th>Measurement (mean±SD)</th>
<th>Pre-tumor</th>
<th>Pre-TAE (day 0)</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume [mL]</td>
<td>84.6±19.9</td>
<td>79.7±13.6</td>
<td>72.4±16.9</td>
<td>79.7±16.0</td>
<td>90.2±20.5</td>
<td>95.7±28.9</td>
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<tr>
<td>Caudal volume [mL]</td>
<td>16.7±5.0</td>
<td>17.4±3.7</td>
<td>16.1±4.3</td>
<td>17.5±3.9</td>
<td>19.2±4.5</td>
<td>20.8±6.1</td>
</tr>
<tr>
<td>Cranial volume [mL]</td>
<td>67.9±16.4</td>
<td>62.4±11.7</td>
<td>56.4±14.2</td>
<td>62.3±14.8</td>
<td>71.0±17.6</td>
<td>75.0±24.2</td>
</tr>
<tr>
<td>Tumor volume [mL]</td>
<td>0.7±0.6</td>
<td>4.66±4.9</td>
<td>4.77±4.7</td>
<td>7.61±6.7</td>
<td>11.30±11.2</td>
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</tr>
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</table>
Tumor response

Tumor volume increased over time in both groups (figure 3A). Considering the separate time points post-embolization, tumor volumes remained stable between day 3 and 7, after which re-growth was seen. The mean volume of the tumor mass after TAE was smaller compared to the sham group, but there was no statistical difference between the groups after 14 days. TGR increased over time in both groups, with a lower trend seen in the TAE-group, although no significant differences were observed between both groups (figure 3B).

Table 2. %CLV (caudal liver volume), %CRLV (cranial liver volume), and TGR (tumor growth rate) before and after TAE

<table>
<thead>
<tr>
<th>CT volumetry</th>
<th>Sham</th>
<th>TAE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 (pre-TAE)</td>
<td>%CLV</td>
<td>18.4±2.0</td>
<td>22.7±0.7</td>
</tr>
<tr>
<td></td>
<td>%CRLV</td>
<td>79.1±2.0</td>
<td>76.6±0.7</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>1.00±0.0</td>
<td>1.00±0.0</td>
</tr>
<tr>
<td>Day 3</td>
<td>%CLV</td>
<td>20.5±2.1</td>
<td>24.4±1.1</td>
</tr>
<tr>
<td></td>
<td>%CRLV</td>
<td>71.7±2.1</td>
<td>70.8±1.7</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>3.4±0.5</td>
<td>7.6±2.7</td>
</tr>
<tr>
<td>Day 7</td>
<td>%CLV</td>
<td>18.8±2.3</td>
<td>25.3±1.3</td>
</tr>
<tr>
<td></td>
<td>%CRLV</td>
<td>64.3±2.2</td>
<td>69.9±2.1</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>9.1±2.4</td>
<td>7.1±2.1</td>
</tr>
<tr>
<td>Day 10</td>
<td>%CLV</td>
<td>19.8±1.7</td>
<td>25.0±0.8</td>
</tr>
<tr>
<td></td>
<td>%CRLV</td>
<td>56.9±1.9</td>
<td>68.4±2.1</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>14.4±3.5</td>
<td>12.0±4.9</td>
</tr>
<tr>
<td>Day 14</td>
<td>%CLV</td>
<td>20.5±1.8</td>
<td>26.6±1.0</td>
</tr>
<tr>
<td></td>
<td>%CRLV</td>
<td>48.8±2.4</td>
<td>64.9±2.5</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>24.1±7.2</td>
<td>18.3±7.7</td>
</tr>
</tbody>
</table>

Figure 1. %CLV data for the sham- and TAE-group. Analyzing the separate time-points, TAE showed significant higher CLV-values on day 7 (p=0.038), 10 (p=0.016), and 14 (p=0.016) compared to the sham-group (panel A). Panel B shows a decrease in %cranial liver lobes in the TAE-group.

Tumor response

Tumor volume increased over time in both groups (figure 3A). Considering the separate time points post-embolization, tumor volumes remained stable between day 3 and 7, after which re-growth was seen. The mean volume of the tumor mass after TAE was smaller compared to the sham group, but there was no statistical difference between the groups after 14 days. TGR increased over time in both groups, with a lower trend seen in the TAE-group, although no significant differences were observed between both groups (figure 3B).
Biochemistry

There was a transient elevation of plasma AST/ALT levels after TAE (figure 4). Also, GGT, LDH and albumin were shortly significantly increased, with peak concentrations on day 1 and 3, returning to normal within 7 days (data not shown).

Histology

Histopathological evaluation of sections of the tumor demonstrated no signs of inflammation. The tumor showed confluencing areas of necrosis and apoptosis. A capsule of fibrotic tissue was seen around the tumor. Although 60-70% of tumor was necrotic, approximately 30-40% of the tumor still contained viable tumor cells, whereas in the sham group no tumor necrosis was apparent. Sections of parenchyma of the embolized cranial liver lobe

Figure 2. There was a strong and significant correlation between total liver volume (TLV) as measured by CT volumetry and actual liver weight determined at sacrifice ($r=0.965$, $p<0.001$). Additionally, caudal liver volume (CLV) as measured by CT volumetry also correlated well with actual caudal liver lobe weight measured after sacrifice ($r=0.905$, $p<0.001$).

Figure 3. Tumor% (panel A) increased significantly after TAE ($^*p<0.05$). %TGR data plotted as a function of time in panel B. TGR increased over time for both groups, without significant differences between groups.
demonstrated normal liver structure without tissue necrosis nor apoptosis, but with minimal portal inflammation. Also, mild sinusoidal dilatation was observed in the cranial liver lobe. In accordance with the volumetric results as mentioned above, the caudal liver lobe contained a significantly higher number of proliferating hepatocytes in the Ki-67 stained slides, compared to the cranial liver lobe. The median number of proliferating hepatocytes per field of view in the cranial liver lobe was 18 (range 9-106), compared to 24 (range 11-77) in the caudal lobe (p=0.004).

Discussion

For patients with primary or metastatic liver tumor who are unresectable due to insufficient FRL-volume, preoperative PVE is a successful technique to induce hypertrophy of the FRL, and to minimize the risk of extensive liver resection. [9,10,11] In recent years, however, an increasing number of articles have been published showing induction of tumor growth as side effect of PVE.[12,13,14] Therefore, TAE alone or sequentially in combination with PVE has been suggested to induce hypertrophy of the FRL, in the meantime preventing tumor growth.[15,16,17] A similar hypertrophy response has also been described after hepatic arterial radioembolization using yttrium-90.[18] Besides destructing the tumor, the radiation treatment causes intrahepatic parenchymal damage, fibrosis and portal hypertension, generating a regeneration response of the untreated, contralateral lobe.

Our study was limited to assessment of the effects of bland hepatic artery embolization on tumor growth and liver regeneration. The technique of TAE, using a transauricular approach, appeared feasible. Only minor spasm, caused by the microcatheter and guidewire, was seen in the small target vessels, but this did not impede the embolization process. In previous experiments, using rabbits, spill of Polyvinyl Alcohol (PVA) particles was seen in non-targeted arterial branches, causing ischemia of the stomach and duodenum (data not shown). Therefore, the present experiments were conducted using a single 2 mm microcoil, resulting in complete occlusion of the tumor feeding arterial branches without damaging the surrounding liver tissue. Although arterial embolization did cause atrophy of
the tumor bearing cranial liver lobes, normal liver parenchymal structure with minimal portal inflammation and mild sinusoidal dilatation in the cranial lobes was seen on the histological investigation.

Despite arterial embolization, increase of tumor volume was seen on CT volumetry, after an initial steady state. Tumor volume was smaller compared to the sham group but this difference was not significant. On histopathological examination however, massive necrosis and apoptosis of the tumor was seen, whereas tumors in the sham group did not show any necrosis or apoptosis. The percentage of necrosis was probably underestimated, as during sacrifice of the rabbits, necrotic parts of the tumor were not included in the histological sections.

Although histologically undamaged parenchyma was seen in the cranial lobe after embolization of the tumor, a significant hypertrophy response was apparent in the caudal, non-embolized lobe. These observations indicate that arterial embolization of only the hepatic arterial branches supplying the tumor, causes significant tumor necrosis and in addition a hypertrophy response of the non-embolized caudal lobe, in the absence of histological damage of the embolized lobe. The findings at CT-volumetry were confirmed by the increased regeneration response observed using Ki-67 staining of histological sections. This is line with the publication of Vogl et al.[15] who already in 1998 described a 10% volume decrease of the embolized lobe after embolization of the right hepatic artery in patients with an initially unresectable cholangiocarcinoma, and a hypertrophy response of the unaffected left lobe, with a mean increase of 37%. In 2011 Denecke et al. [19] compared TAE with PVE in patients with cholangiocarcinoma and also described a hypertrophy response after arterial embolization. The increase of the FRL-volume, however, was less than that after PVE.

It is believed that the regeneration response after portal vein embolization is not only caused by the hemodynamic changes after occlusion of part of the portal system, but is also set off by upregulation of several humoral mediators (HGF, TNF-α, TGF-α, IL-6, insulin) released after hepatocellular damage, causing a regeneration response that leads to hypertrophy of the non-embolized lobe.[19] Although the exact mechanism is not specifically investigated, it is assumed that the hypertrophy response after arterial embolization is mediated by the same pathways.[20] The fact that PVE has a superior hypertrophy response compared to arterial embolization can be explained by its greater effect on overall hepatic blood perfusion. The question remains however, whether the regeneration response of the caudal lobe is caused only by the damage incurred in the tumor after embolization. On basis of the results of this study, this seems highly suggestive.

Conclusion

The transauricular approach of hepatic artery embolization is a feasible technique in a rabbit VX2 tumor model. Super selective, bland arterial coil embolization causes massive necrosis of the tumor, despite increase of volume on CT-volumetry. Atrophy of the tumor bearing
liver lobe is seen after arterial embolization of the tumor with a concomitant hypertrophy response of the non-embolized lobe, despite absence of histological damage of the tumor-surrounding liver parenchyma.
References