Cytokines pattern after surgical radiofrequency ablation of liver colorectal metastases

Serge EVRARD (1), Christine MENETRIER-CAUX (2), Cathy BIOTA (2), Véronique NEAUD (3), Simone MATHOULIN-PELLISSIER (4), Jean-Yves BLAY (2), Jean ROSENAUM (3)

(1) Institut Bergonié, Digestive Tumours Unit, Bordeaux ; (2) Centre Léon Bérard, Cytokines Laboratory, Lyon ; (3) INSERM, E362, Université Victor Segalen Bordeaux 2 ; (4) Institut Bergonié, Department of Biostatistics, Bordeaux.

Introduction

Radiofrequency ablation is a local ablative technology initially designed to destroy tumours percutaneously [1]. More recently, it has been proposed as a surgical tool. For some teams, surgical radiofrequency ablation (SRFA) is indicated when the tumours are not resectable [2, 3], as a single treatment or as a complement to resection. For others [4-6], it may be applied curatively for small metastases especially when deeply located within the liver parenchyma the latter of which can therefore be spared.

Hepatocytokines involving from 30 to 80% resected volume [7] and portal embolization [8] increase the blood levels of a panel of cytokines involved in the liver regeneration process. On the other hand, cryoablation [9] and even simple laparotomy [10] also induce the same circulating level of cytokines without changes in liver volume. Little is known about SRFA which destroys mainly neoplastic tissue and damages only a small margin of surrounding healthy liver. Recently, Ng et al. [11] reported in the swine a systemic response of RFA significantly less severe than those of cryoablation but comparable with hepatectomy. The aim of this study was to evaluate the serum pattern of evolution of cytokines after SRFA of colorectal metastases in order to evaluate, in intention-to-treat, the inflammatory stress secondary to this procedure in man.

Patients and methods

The protocol of the present study was designed with the collaboration of the Department of Biostatistics of our institution and approved by the Institutional Review Board.

Reprints: S. EVRARD, Université Victor Segalen-Bordeaux 2, Institut Bergonié, 229, cours de l’Argonne, 33076 Bordeaux.
E-mail : evrard@bergenie.org

SUMMARY

Aims — The aim of this study was to evaluate the serum pattern of cytokines evolution after surgical radiofrequency ablation (SRFA) of colorectal metastases.

Methods — Metastases of ten non consecutive patients were destroyed by radiofrequency ablation without concomitant resection after a complete surgical procedure including a laparotomy, a peritoneal examination, liver mobilisation and liver ultrasound. Serum levels of IL-6, TNFα, HGF, VEGF, bFGF, TGFβ1 and CRP were assessed by ELISA assays at different time points.

Results — TNFα and bFGF remained undetectable. IL-6 peaked at 3 hours and remained elevated during the entire study period. HGF increased by three-fold by Day 1 then decreased until Day 7 where it was still twice its baseline level. VEGF level increased from Day 5 onward. TGFβ1 did not show significant variations. CRP was increased throughout the study.

Conclusions — In contrast with cryotherapy, SRFA does not lead to high serum TNFα suggesting a better tolerance. Nevertheless high IL-6, HGF and VEGF serum levels are characteristic of a general inflammatory stress which should be taken into account.

RÉSUMÉ

Buts — Étudier le profil d’évolution de plusieurs cytokines après ablation chirurgicale par radiofréquence de métastases d’origine colorectale.

Méthode — Les métastases de dix malades non consécutifs ont été détectées par radiofréquence sans résection parenchymateuse associée au cours d’une intervention par laparotomie incluant une exploration péritonéale, une mobilisation du foie ainsi qu’une échographie peropératoire. Les taux sériques d’IL-6, TNFα, HGF, VEGF, bFGF, TGFβ1 et CRP ont été dosés par système ELISA au cours de la première semaine postopératoire.

Résultats — TNFα et bFGF sont restés indétectables tout au long de l’étude. L’IL-6 a présenté un pic à 3 heures et est resté élevé durant toute l’étude. Le taux d’HGF a été multiplié par trois dès le premier jour puis a décru jusqu’au 7e jour où il était encore double de son taux initial. Le taux de VEGF s’est élevé à partir du 5e jour. TGFβ1 n’a pas montré de variations significatives. La CRP était élevée durant toute l’étude.

Conclusion — Contrairement à la cryothérapie, les radiofréquences à ciel ouvert n’induisent pas d’élévation du TNFα, suggérant une meilleure tolérance. Néanmoins, les taux d’IL-6, HGF et VEGF sont caractéristiques d’un stress inflammatoire proche de celui rapporté après hépatectomie.
Tableau I. – Patients characteristics.

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Age (years)</th>
<th>Number of Metastases (size in mm)</th>
<th>Total energy Delivered (J)</th>
<th>Duration of procédure (min)</th>
<th>Pringle manoeuvre (duration in min)</th>
<th>Duration of follow up (months)</th>
<th>Follow up</th>
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<td>48</td>
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<tr>
<td>2</td>
<td>73</td>
<td>5 (10, 13, 10, 10, 22)</td>
<td>157 420</td>
<td>255</td>
<td></td>
<td>22</td>
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<tr>
<td>3</td>
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<td>3 (6, 6, 6)</td>
<td>58 000</td>
<td>360</td>
<td></td>
<td>21</td>
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<tr>
<td>4</td>
<td>55</td>
<td>3 (35, 40, 40)</td>
<td>304 560</td>
<td>240</td>
<td>+ (77)</td>
<td>11</td>
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<tr>
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<td>173 670</td>
<td>240</td>
<td>+ (14)</td>
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<td>152 241</td>
<td>240</td>
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<td>+ (31)</td>
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<td>71</td>
<td>4 (25, 5, 10, 6)</td>
<td>123 565</td>
<td>195</td>
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<td>10</td>
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<td>49 345</td>
<td>195</td>
<td>+ (6)</td>
<td>24</td>
<td>Alive without cancer</td>
</tr>
</tbody>
</table>

Blood sampling and processing

Blood samples from the patients enrolled in this study were collected through of a venous catheter before treatment (Day D₁ and D₀ before intervention) and after the end of the treatment (D₀ +3h, D₁, D₂, D₃, D₅ and D₇) in the morning to avoid nychthemeral variations. After centrifugation, serum samples were aliquoted and immediately stored at -80°C until assay.

Interleukin 6 (IL-6), tumor necrosis factor α (TNFα), transforming growth factor β (TGFβ), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF) serum levels were determined using specific commercial quantitative sandwich enzyme immunoassay technique (R&D Systems). The sensitivity of these assays, defined as the lowest cytokine concentration significantly different from the zero standard with a probability of 95% are 7 pg/mL, 3 pg/mL, 5 ng/mL, 40 pg/mL, 0.7 pg/mL and 4.4 pg/mL, respectively.

C-reactive protein (CRP) determinations were performed on a Synchro Clinical automatized System (CX5 CE Beckman) with CRP microlatex reagent (Sobiola).

Statistics

Serum levels of TGFβ, VEGF, HGF, IL-6 and CRP are presented as box-and-whisker plots showing median value and interquartile range (IQR). Missing data (MD) were replaced by the mean of each serum level (1 MD for D₁; 1 MD for D₀ +3h; 2 MD for D₅). The Levene test was performed to detect inhomogeneity of variance and if detected, the data were log-transformed (base 10) in order to stabilise variances (HGF, VEGF, IL-6, CRP). Then, for each cytokine, statistical analyses were performed using repeated measure one-way ANOVA. Statistical analyses were carried out using the statistics package SPSS v12.0 (SPSS Inc., Chicago, IL, USA). When comparing multiple measures, a P-value less than 0.01 was considered to be statistically significant.

Results

Postoperative courses were uneventful excepted for one patient who presented a fever treated by antibiotics.

At baseline, VEGF, TGFβ, and HGF concentrations were 231.8 pg/mL (range: 124.7-522.5 pg/mL), 46.2 ng/mL (range: 16.9-98 ng/mL) and 1257 pg/mL (range: 654-1612 pg/mL), respectively. For IL-6, TNFα, and bFGF, basal levels were below detection thresholds. Sera of patients were tested for their cytokines content over 7 days. TNFα was undetectable at every time point during the follow-up. Values for bFGF
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Fig. 1 – Variation of cytokine levels before and after SRFA treatment. The top and bottom of each box represent the 75th and 25th percentiles, respectively. The horizontal line within the boxes represents the 50th percentile (median) and the end of each “whisker” the 10th and 90th percentiles (1a); Serum levels of interleukin 6 (IL-6) (1b); Serum levels of hepatocyte growth factor (HGF) (1e); Serum levels of vascular endothelial growth factor (VEGF) (1d); Serum levels of transforming growth factor β1 (TGF-β1) (1e); Serum levels of C reactive protein (CRP). (1f).

Variation des taux de cytokines sériques avant et après traitement par radiofréquence peropératoire. Le sommet et la base de chaque boîte représentent respectivement le 75e et le 25e percentiles. La ligne horizontale contenue dans chaque boîte représente le 50e percentile (médiane) et les extrémités de chaque “moustache” les 10e et 90e percentiles (1a) ; Taux sériques d’interleukine 6 (IL-6) (1b) ; Taux sériques d’hepatocyte growth factor (1e) ; Taux sériques de vascular endothelial growth factor (1d) ; Taux sériques de transforming growth factor β1 (1e) ; Taux sériques de protéine C réactive (1f).
were also mostly below the level of detection. Whereas IL-6 levels were below the threshold for detection before surgery, we observed a rapid increase in IL-6 serum concentration in the hours following the end of the treatment (figures 1a and 1b). The IL-6 level decreased progressively but remained detectable until the end of the follow-up, in contrast to that observed at baseline. The changes in IL-6 serum levels were highly significant (P < 0.0001).

SRFA treatment also induced a rapid increase of the HGF serum content that reached three times the baseline level at day 1 (figure 1c). HGF concentration decreased from day 2 until day 7 but remained two fold higher than the baseline value (P < 0.0001). In contrast, VEGF followed different kinetics with a late increase at day 5 that persisted at day 7 (figure 1d). These variations were also highly significant (P < 0.0001). Finally, although TGFβ1 levels tended to progressively decrease until day 3 (figure 1e), variations did not reach statistical significance. CRP levels rose from day 1 and remained very high at the end of the follow-up period (figure 1f).

**Discussion**

All invasive surgical procedures induces the release of a variety of cytokines leading to a general inflammatory stress. This stress response may be implicated in positive processes like the healing of the injured tissues but could also result in the stimulation of tumor cell growth. Sometimes it may lead to severe postoperative disorders like in the case of shock induced by cryotherapy.

A high CRP level, a marker of inflammation, was observed at all time points during the study. However, TNFα, a major proinflammatory mediator remained undetectable. The lack of increase of TNFα in serum was also observed in another series of patients undergoing SRFA [12] as well as after partial hepatectomy [13-15]. This is in contrast with liver cryotherapy which induced a TNFα increase proportional to the degree of hepatic freezing [16]. As TNFα is known to be a major mediator of shock, these data suggest that SRFA may be safer in this respect than cryotherapy for the treatment of liver tumours.

One of the major concerns of surgical procedures for liver metastasis is that the procedure itself may stimulate the growth of dormant metastases through the induction of the liver regeneration process. Liver regeneration is orchestrated by a complex system involving many cytokines and growth factors using both paracrine and endocrine pathways. A simplified scheme [17] suggests that hepatocytes need to be primed for replication through the consecutive action of TNFα and IL-6. They then become responsive to mitogenic growth factors such as HGF or TGFβ. In addition, VEGF also plays a major role in the regeneration of the liver vasculature, and is also involved in hepatocyte replication. On the other hand, TGFβ1 is involved in the arrest of regeneration.

We found a rapid and sustained increase in serum IL-6 in patients undergoing SRFA. IL-6 is a multifunctional cytokine produced as a part of the acute-phase response in tissues exposed to non specific insults. As already mentioned, IL-6 appears to be essential for the priming of hepatocytes as demonstrated in knockout mice for the IL-6 gene where hepatocyte DNA synthesis is impaired, leading to liver failure [18]. An increase in serum IL-6 has been previously reported in humans after partial hepatectomy [13, 19] or cryosurgery [9]. In the single published study dealing with serum cytokines after SRFA [12], IL-6 reached a 3-fold peak at 24 hours and decreased at 48 hours. In experimental liver regeneration, IL-6 induction is dependent on TNFα signalling, as demonstrated using TNF Receptor-1 knockout mice [20]. However, IL-6 supplementation can compensate defects in TNFα signalling [20], thus suggesting that in our patients, despite the absence of a TNFα serological response, the raised IL concentration is a significant indicator for regeneration.

We also found increased HGF serum levels following SRFA. This is similar to results reported by De Jong et al. [9] after cryo-therapy or partial hepatectomy [10]. Classical sources of HGF in the liver are Kupffer or hepatic stellate cells. In the RFA setting, it could also originate from damaged endothelial cells [21].

The delayed increase in VEGF expression is concordant with results observed following partial hepatectomy in animals where it is known to be important for both neoangiogenesis [22, 23] and hepatocyte regeneration [24]. In the SRFA setting, VEGF expression may be under the direct control of cytokines such as IL-6 [25] or HGF [26] or may be related to the tissue hypoxia generated by RFA. We did not find significant modifications in the serum concentration of another angiogenic factor, bFGF, which is compatible with the fact that its role does not seem to be essential in the regeneration process [27].

In conclusion, our results in this intention-to-treat series testify that the overall procedure of SRFA appears to induce less inflammation than cryotherapy performed for the same indications. Given the pattern of serum cytokines observed, surgical use of radiofrequency induces an inflammatory stress near to these reported in the literature for parenchymal resection [10, 13, 14]. Thus, our data can not however exclude the possibility that RFA may also stimulate the growth of dormant metastases. Longitudinal studies are warranted to address this issue.

**REFERENCES**

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