Post-Occlusive Reactive Hyperemia in Basal Cell Carcinoma and Its Potential Application to Improve the Efficacy of Solid Tumor Therapies

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Tumor hypoxia is a hallmark of malignant tumors, and is a major factor in the resistance to anti-cancer therapies, particularly radiotherapy. Indeed, tumor blood flow often fluctuates, and thus the oxygen supply is often reduced, thereby inducing tumor hypoxia. We decided to explore whether post-occlusive reactive hyperemia, a physiological reaction known to occur in normal tissues, could be induced through a malignant tumor, basa cell carcinoma (BCC), in which angiogenesis occurs, as in all malignant tumors. Skin blood flow was measured in twelve patients with BCC, using Laser Speckle Contrast Imaging to determine BCC perfusion after three minutes of vascular occlusion, induced by limb tourniquet for limb tumors (4 BCC), and/or by clamping the pedicle of a skin flap with the BCC at its center, for other tumor locations (12 BCC). We demonstrated for the first time that post-occlusive reactive hyperemia occurs in malignant tumors in humans. BCC perfusion curves were similar to those of healthy skin, characterized by a peak of hyperemia after reperfusion followed by a progressive return to the pre-occlusion perfusion level. Induction of post-occlusive reactive hyperemia in malignant tumors is therefore a novel investigational approach that could lead to a new adjuvant tool to increase the efficacy of chemotherapy and radiotherapy, respectively through the synchronized temporary increase of tumor perfusion and oxygenation.

Keywords: basal cell carcinoma; laser speckle contrast imaging; limb tourniquet; post-occlusive reactive hyperemia; random-pattern skin flap

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al. 2009), and since PORH is an ubiquitous reaction, PORH could theoretically be used to treat all tumors, regardless of their location, and to the reactivity of the intra-tumoral vessels following vascular occlusion.

We demonstrated for the first time the occurrence of PORH in basal cell carcinoma (BCC), the most frequent human malignant tumor (Crowson 2006), as a model of tumor microcirculation. BCC was selected, because it exhibits angiogenesis similar to other malignant tumors, with increased density, tortuous dilated vessels (Bedlow et al. 1999; Newell et al. 2003; Stanton et al. 2003) and elevated blood flow (Stücker et al. 1999; Stanton et al. 2003). In addition, the skin blood flow over this superficial tumor can be easily assessed using laser speckle contrast imaging (LSCI) (Roustit et al. 2010; Rousseau et al. 2011), a new and accurate technology for skin blood flow assessment, enabling non-invasive, two-dimensional flux recording at a high sampling rate.

**Materials and Methods**

**Study population**

Inclusion criteria were: age greater than 18 years and biopsy-proven BCC. Exclusion criteria were: BCC bone invasion, and counter-indication to tourniquet for limb BCC. Institutional (Commission des Études et de la Recherche Clinique) and National (Comité de Protection des Personnes) Clinical Research Ethics Committee specifically approved this study, and patients provided written informed consent before participation in the study. This study is registered as clinical trial with the National Cancer Institute: http://clinicaltrials.gov/ct2/show/NCT01455363. Twelve patients with sixteen BCCs were included in the study (Table 1).

**Laser Speckle Contrast Imaging (LSCI)**

Skin blood flow was recorded consistently throughout the experiment, using a high frame rate laser speckle contrast imager (PeriCam PSI System, Perimed). The laser wavelength was 785 nm. The laser head was placed 20 cm above the skin. The image acquisition rate was 8 s⁻¹.

**Data acquisition conditions**

None of the patients smoked. Experiments were performed in an operating room (the temperature was controlled at 23°C), with the patient in a horizontal position, starting after a 30-minute period of acclimatization.

**Experimental protocols**

Two principal protocols were applied. The tumor-bringing skin flap protocol (corresponding to the surgical cutting of a pedicled skin flap with the BCC at its center) was applied alone for 12 BCC, and limb tourniquet protocol was applied alone for 4 BCC. One BCC (BCC n°3) underwent the two protocols consecutively (first limb tourniquet then skin flap protocol).

**A. Tumor-bringing skin flap protocol (Fig 2) (13 BCC)**

The skin flap design was a 4-mm margin around the BCC, and using a pedicle of 1⁄3 of the margin perimeter. A black marker permitted margin visualization and Region of Interest (ROI) selection on laser imaging.

- In case of local anesthesia (9 BCC), 1% lidocaine was used without adrenaline to avoid any vasoconstrictor effect on skin vessels. Eight aliquots of 0.5 ml of 1% lidocaine were injected into the margin.
Post-Occlusive Reactive Hyperemia in a Tumor

Table 1. Summary of the patients, basal cell carcinomas and experiment types.

<table>
<thead>
<tr>
<th>Patient n°</th>
<th>BCC n°</th>
<th>Age</th>
<th>Sex</th>
<th>Localization</th>
<th>BCC Histology</th>
<th>BCC Dimensions (length × width (mm))</th>
<th>Test Type</th>
<th>Flap Anesthesia Type</th>
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<td></td>
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<td>Tourniquet</td>
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<td>1</td>
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<td>61</td>
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<td>Nose point</td>
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<td>3</td>
<td>86</td>
<td>M</td>
<td>Right leg</td>
<td>S</td>
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<td>M</td>
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<td>Right temple</td>
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<td>4</td>
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</table>

I: Infiltrant (i.e., dermic proliferation of diffuse small tumor cell clusters), N: Nodular (i.e., infiltrant histology with big clusters of tumor cells and the clinical shape of a nodule), S: Superficial (i.e., tumor cell clusters adjoining the epidermic basal membrane), SCL: Sclerosing (i.e., continuous dermic tumoral proliferation into the sclerotic tumor stroma)

Fig. 2. Tumor-bringing skin flap protocol photographs of a right shoulder BCC (patient n°7, BCC n°11).

A. After basal flow measurement, the skin flap is raised through hypodermis, and only the flap pedicle is not cut. The deep face of the skin flap is represented by the dotted line. B. After fixation of the skin flap to the peripheral skin by three surgical sutures (three arrows) and measurement of baseline flow, five minutes after initiation of flap, a bulldog clamp (thick arrow) was used to occlude the BCC vascular supply by pinching the skin flap pedicle for three minutes (double head arrow). Ischemic BCC (dotted arrow) is at the center of the skin flap. After the clamp is retrieved, flow is measured for 5 minutes.

line. Five minutes after the injection, the skin flap was raised. We cut the skin flaps to the depth of the hypoderm, to avoid vascular dermic plexus lesions. Flaps were fixed to the skin by suture points to avoid retraction. Five minutes after beginning the flap raising procedure, the flap pedicle was clamped for 3 minutes with a Bulldog clamp, and then retrieved. We selected the duration of 3 minutes of vascular occlusion, because it is most often used in clinical experiments of PORH, and because it provided the highest post-occlusive...
peak hyperemia in normal skin (Betik et al. 2004). Efficient occlusion, and immediate reperfusion was controlled by LSCI. After 5 minutes of measurement, the flap was removed.

- In case of general anesthesia (4 BCC), no local anesthesia by lidocaine was used. Indication for general anesthesia was unrelated to our protocol, and was due to necessity of surgical reconstruction just after BCC removal, patient anxiety, and/or more than two BCC.

B. **Limb tourniquet protocol (4 BCC)**

An upstream BCC tourniquet (arm or tight) was occluded for 3 minutes at 50 mmHg greater than the systolic arterial pressure. The tourniquet was then opened, and perfusion was measured for 5 minutes.

### Data analysis

Data were digitized, stored on a computer, and analyzed off-line with signal processing software (PimSoft for LSCI measurements; Perimed). The software expresses recorded values in perfusion units. The mean ROI surface perfusion values were automatically calculated by PimSoft, based on the individual pixels values for each perfusion image, and that for the whole TOI (Time Of Interest) periods.

1. **ROI selection protocol**

ROI lines were drawn using the signal processing software (PimSoft, Perimed).

The BCC ROI was visible as a well-defined hyperperfused zone, corresponding to the macroscopically visualized BCC, inside the resection margin line marked with a black pen. Healthy skin ROI was the zone between the margin line and the BCC ROI line. Distal and proximal flap ROI were the healthy skin between 10 hours and 2 hours, and 4 hours and 8 hours, respectively (with the flap pedicle center at 6 hours).

2. **TOI selection**

TOI periods have a duration of 10 second, and we checked that patients remained motionless during those periods to avoid laser speckle signal artefacts. TOI were placed at each experimental step end, and every 30 seconds, starting just after release of BCC vascular occlusion, during the 5 minute study period. The percentage of the flux value variation between pre- and post-lidocaine or flap raising was calculated as: (Post - Pre)/Pre values. The hyperemia peak was selected as the highest TOI value, measured only one time after reperfusion. Thus, we avoided the selection of just an instantaneous peak-choice which would have been subjective-, because small movement artefacts or cardiac systole induced perfusion peaks could provide confusion. Hyperemia percentage = (Hyperemia Peak – Preclamping value) / Preclamping value. Finally, the BCC perfusion value after 5 minutes of measurements following release of occlusion always reached the initial level.

### Statistical analysis

Data were analyzed using R software. Mann-Whitney and Kruskal-Wallis tests were used for comparisons of the medians between two groups or more. Wilcoxon’s matched pairs, signed-ranks and Friedman’s tests were used for paired comparisons. Spearman correlation test was used to correlate perfusion variables. Significance was accepted at $p < 0.05$. Results are expressed as the median and interquartile in parentheses.

### Results

**BCC exhibit elevated basal perfusion compared to healthy skin perfusion, reflecting angiogenesis in BCC vessels**

Basal BCC perfusion (16 BCC) was more than two fold that of healthy skin perfusion (134.1 Perfusion Units [PU] (49.7) vs. 66.1 (27.8); $p < 0.05$), in accordance with the literature (Stücker 1999). Increased tumor microvascular density and vessel diameter compared with healthy skin likely explains the hyperperfusion as a result of tumoral angiogenesis, which occurs in BCC, as in all malignant tumors (Bedlow et al. 1999; Newell et al. 2003; Stanton et al. 2003).

**BCC perfusion increased secondary to peri-tumoral lidocaine injection**

BCC supplying vessels and/or intra-tumoral vessels were reactive to lidocaine (in the 9 BCC of the tumor-bringing skin flap protocol). Five minutes after the injection of lidocaine, the increase was 73% (50.1%) in healthy skin vs. 34.4% (27.2%) in BCC ($p < 0.05$). The well-known skin vasodilatory effect of the lidocaine was thus logically predominant in injection site of healthy skin, which is known to increase its flow rapidly after lidocaine injection (Ghali et al. 2008). The increase in BCC flow is thus a passive increase in response to vasodilatation of tumor supplying vessels, with or without active mechanism by tumor proper (intra-tumoral) vessels vasodilatation.

**Skin flap raising induced an increase in BCC perfusion under general anesthesia, but a decrease after lidocaine anesthesia**

Five minutes after flap raising start, in the general anesthesia group (4 BCC), an increase in healthy skin and BCC perfusion was observed: +19.6% (23.2%) vs. +22.5% (27%) (non-significant; NS), that could be explained by a myogenic reflex (vasodilatation secondary to the fall in intravascular pressure in the skin flap) (Schubert and Mulvany 1999), with preferential vasodilatation and flow increase in proximal part of flap (93% (54.2%)) and BCC, at the expense of the too far distal part of flap [−25.5% (5%)] ($p < 0.05$). Thus, it expressed a new sharing of skin flap regional flow, with extremity flow fall and proximity flow rise, which has already been observed in rat random-pattern skin flap model (Marks 1985).

By contrast, the preliminary peri-tumoral lidocaine injection, through induced vasodilatation, seemed to diminue the vasodilatatory reserve thus the myogenic reflex capacity to adapt BCC perfusion after flap raising. Indeed, BCC perfusion fell by 30.7% (44.1%) ($p < 0.05$).

**Detection of PORH in all BCCs**

PORH after three minutes of vascular occlusion was evident in all sixteen BCC (Table 2). Classical aspect of perfusion curves, described for healthy tissues in literature, was measured in BCC, with a relatively early perfusion...
Table 2. Basal cell carcinoma laser speckle perfusion values (values are in Perfusion Units, except for the percentage data).

<table>
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<tr>
<th>Experiment Type</th>
<th>BCC n°</th>
<th>Basal Perfusion</th>
<th>Post-lidocaine Perfusion</th>
<th>Post-lidocaine VP (%)</th>
<th>Post-“flap raising” Perfusion</th>
<th>Post-flap VP (%)</th>
<th>Post-occlusive Hyperemia Peak</th>
<th>Post-occlusive Hyperemia Percentage</th>
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<td>Tourniquet</td>
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<td>143.82</td>
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<td>169.19</td>
<td>137.9</td>
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<tr>
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<td>5</td>
<td>81.22</td>
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VP: variation percentage of perfusion ((Post-Pre)/Pre values). The time interval between basal perfusion, post-lidocaine and post-flap raising measurement is 5 minutes.

Fig. 3. Laser Speckle Contrast Images of Post-Occlusive Reactive Hyperemia in BCC.
These two cases (black arrows, photographs on the left) represent the two experimental protocols: limb tourniquet (A) and skin flap (B). (A) Left Foot BCC (patient n°3, BCC n°5) surrounded by the resection margin (black line), before (left image), during (center image), and 30 seconds after 3 minutes of ischemia (right image). Previous central BCC biopsy scar (red arrow); tumor hyperemia of 69.76% (perfusion scale red color intensity increase). (B) Left Shoulder BCC (patient n°4, BCC n°8), under general anesthesia. The flap was raised around the BCC, and the pedicle clamped by a Bulldog (red asterisk). Before (left), during (center), and 30 seconds after (right image) 3 minutes of clamping. Tumor hyperemia percentage: 16.2%.
peak after reperfusion, followed by a progressive decrease of BCC perfusion, until return to its pre-occlusion value. Median hyperemia percentage was 14.9% (34.5%) for BCC, and 10.8% (26.7%) for healthy skin (NS), with a time to peak (i.e., interval between declamping and perfusion peak), of 80 (157.5), and 65 (150) seconds, respectively (NS). BCC post-occlusive hyperemia peak values were significantly greater than the perfusion values just before vascular occlusion ($p < 0.05$).

The BCC hyperemia percentage was 57.9% (18.5%) for limb tourniquet group (4 BCC) (Fig. 3A), 16.4% (1.6%) in the skin flap sub-group under general anesthesia (4 BCC) (Fig. 3B), and 11% (10.4%) in the skin flap sub-group under lidocaine anesthesia (9 BCC) (NS). BCC typical post-occlusive reactive hyperemia (Fig. 4) was visually evident in the laser speckle contrast imaging perfusion curves from the limb tourniquet protocol, due to the high level of hyperemia percentage in this group.

**Lidocaine decreased the BCC vasodilatatory reserve**

Lidocaine appeared to decrease BCC hyperemia percentage [11% (10.4%)] compared to the non-lidocaine group (4 limb tourniquet BCC + 4 general anesthesia BCC): 17.4% (41.6%) ($p < 0.05$). PORH consists in a distal bed vasodilatation (Wood et al. 1955) principally induced by ischemia-induced vasodilators synthesis (Tóth et al. 2007) and myogenic response (Schubert and Mulvany 1999). Thereby, pre-occlusion vasodilatation induced by lidocaine decreased the vasodilatatory reserve of BCC supplying vessels and/or BCC vessels themselves, thus resulting in decreased PORH.

It is also coherent with the higher hyperemia percentage in the limb tourniquet BCC (57.9%) compared to tumor-bringing skin flap sub-group under lidocaine anesthesia (11%), although non-significant.

**Most perfused BCCs exhibit a lower degree of hyperemia**

Pre-occlusion perfusion, inversely related to vascular resistance (Pressure = Flow × Resistance), correlated negatively (Spearman correlation test, $p < 0.05$) to PORH (Fig. 5), synonymous to decreased vasodilatatory reserve in hyperperfused BCC, corroborating the previous result of impact of lidocaine on hyperemia. Thus, it appears that BCC supplying vessels and/or BCC intra-tumoral vessels likely exhibited a vasodilatatory reserve that was inversely proportional to their initial diameter.

**Discussion**

Tumor perfusion-mediated hypoxia is one of the two principal causes of tumor oxygen supply deficiency along with diffusion-mediated hypoxia (Thomlinson 1977; Vaupel 1977; Vaupel et al. 1989; Hockel and Vaupel 2001). Regardless of basal tumor flow level, blood flow is heterogeneous through tumor, and cycling hypoxia into tumor occurs, with a negative impact on therapies, particularly radiotherapy that is highly dependent on the tumor partial oxygen pressure only at the time of radiation (Gray et al. 1953; Roots and Smith 1974). Thus, overcoming this problem by modulation of tumor perfusion is necessary, to improve the efficacy of current therapies. Additionally, access of anti-tumoral agents into less perfused tumor parts (Peterson 1991; Fukumura and Jain 1998; Padera et al. 2004) is essential to all therapies.

To the best of our knowledge, this is the first report to demonstrate post-occlusive reactive hyperemia in human tumors. Although the topography of perfusion variation into the BCC was not studied because it is impossible to
precisely standardize the ROI selection protocol into the BCC surface, due to their small size and heterogeneity of their shape, we demonstrated coherent findings of the dilatory vascular reactivity of BCC supplying vessels and/or BCC intra-tumoral vessels to lidocaine, skin flap raising and temporary vascular occlusion. The vasodilatatory reserve of BCC vessels (corresponding to the PORH level) correlated inversely with the initial level of BCC vasodilatation (i.e., BCC perfusion; Fig. 5), confirmed by the greater degree of BCC hyperemia in the lidocaine group [17.4% (41.6%)], compared with the non-lidocaine group [11% (10.4%)].

The experimental protocol was imperfect, firstly due to the low number of limb BCC patients (less than 15% of BCC patients in the general population (Cigna et al. 2011), which was certainly the cause of the lack of any significant difference in the hyperemia percentage between the experimental groups. Second, the small number of patients did not allow us to discriminate a difference (if existing) between BCC and healthy skin reactivity to vascular occlusion. However, high level PORH was clearly demonstrated in the tourniquet group, the only realistic situation, which will be similar to that used in the clinical therapeutic use of the concept of post-occlusive reactive hyperemia in a tumor, regardless of the nature of the tumor. Moreover, measurement of perfusion TOI (10 seconds periods), every 30 seconds after release of occlusion, avoided subjective selection and overestimation of the flow peak, and at least allows for a more realistic assessment of the hyperemia, but even underestimate its level. Indeed, the measured healthy skin median hyperemia percentage of 65.6% (non significantly different from the BCC 57.9% median percentage) is less than the typical level of hyperemia percentage of 200% after 3 minutes of vascular occlusion described in literature (Wood et al. 1955; Marcus et al. 1981; Gourley and Heistad 1984; Roustit et al. 2010; Rousseau et al. 2011).

We did not explore the particular response of the two vascular beds of the tumor supplying vessels and intra-tumoral vessels, since laser speckle imaging does not provide such assessment of the microvasculature. Although reactivity of the BCC supplying vessels (contained in healthy skin) was certain (healthy skin and BCC reacted identically during lidocaine injection, flap raising and temporary vascular occlusion), reactivity of the intra-tumoral vessels to temporary vascular occlusion remains an important question for future investigation using video-capillaroscopy, because intra-tumoral reactive vasoconstriction might have impaired the effect of PORH in tumor supplying vessels during tumor perfusion, as opposed to the vasodilatation or complete passivity of intra-tumoral vessels. However, the general immaturity of intra-tumoral vessels of malignant tumors with almost maximal vasodilatation and low vasodilatory reserve (Suzuki et al. 1981, Peterson 1991, Fukumura and Jain 1998, Vaupel et al. 1998, Thews et al. 2000, Isenberg et al. 2008, Sonveaux et al. 2009) should render those tumors vessels principally unreactive to temporary occlusion. Thus, PORH will be efficient through tumor upstream normal vessels dilatation.

Tumor PORH is the first and only one concept proposed for selective tumor perfusion increase, and thus has
the potential to avoid general side effects, and impairment of local effects linked to the pharmacological approach. Second, it is also the only one rapidly and totally reversible concept that permits repetition -as frequent as needed-, and synchronization of tumor radiotherapy to peak hyperemia, or to repeat periodic occlusion of the tumor supplying vessels during chemotherapy injection (by using flow repayment/debt positive ratio, characteristic of PORH), to improve chemotherapy intra-tumoral perfusion. Third, tumor PORH theoretically has a local potential for modulation, since hyperemia peak and duration are proportional to ischemia duration (Betik et al. 2004). For these three reasons, our results on BCC, although studied as a model of tumor microcirculation, bring a particularly broad and hopefully significant finding, above all in the context of modern endovascular therapy, which is a non-invasive manner of inducing tumor vascular occlusion.

Conclusion

Intratumoral and/or BCC supplying vessels have a vasodilatoratory capacity, and responded with PORH following temporary ischemia with near 60% median hyperemia vasodilatatory capacity, and responded with PORH follow-

Our results and develop a future therapeutic approach. Lymphatic mechanisms, above all intra-tumoral vessel reactivity, must be understood and used clinically for tumor PORH. Others authors did not have any conflict of interest.

Conflicts of Interest

Acknowledgments

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References


Post-Occlusive Reactive Hyperemia in a Tumor


