

SHORT REPORT

EVALUATION OF THE ABILITY OF DIGITAL INFRARED IMAGING TO DETECT VASCULAR CHANGES IN EXPERIMENTAL ANIMAL TUMOURS

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Infrared imaging has frequently been used in the past to detect changes in skin surface temperature associated with breast cancer. Usually a 1–2°C elevation in skin surface temperature is observed at the tumour periphery, and it has been proposed that this change is due to hypervascularity resulting from tumour-associated angiogenesis. In our study, we used the rat mammary adenocarcinoma 13762 MAT, a tumour that has been used to identify antiangiogenic drugs, to investigate whether infrared imaging can detect angiogenesis in malignant tumours. If successful, it was hoped that this technique would represent a simple, noninvasive, procedure for monitoring the activity of antiangiogenic drugs. It was found that, unlike breast cancer patients, no tumour-associated increase in skin surface temperature was observed, but a constant and highly significant reduction in temperature was noted that was independent of tumour size and was produced by relatively small tumours (≥ 0.5 cm in diameter). The explanation for this effect is unclear but it may be due to the poorly vascularised nature of rapidly growing tumours. Nevertheless, our study indicates that the peripheral temperature elevation reported in breast cancer patients is unlikely to be due to hypervascularity resulting from tumour-induced angiogenesis. An alternative explanation is that the temperature increase is due to a chronic inflammatory response around developing breast tumours. With increasing evidence that inflammation can enhance tumour growth and is associated with a poor prognosis, this suggestion implies that infrared imaging may have considerable prognostic value.

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Tumour-associated changes in skin surface temperature, revealed by infrared imaging, have been noted in breast cancer patients since the early 1960s.¹ In fact, for a number of years, infrared imaging, also termed infrared thermography, was used either as an alternative or to complement mammography for breast cancer screening. The procedure, however, fell out of favour as a breast cancer screen more than 20 years ago.^{2,3} The failure of the technique has been attributed to difficulties in interpreting the thermal images and the use of nondigital imaging devices rather than the approach being theoretically flawed.^{2,3} In fact, the profound vascular changes that occur at sites of tumour growth^{4–7} would be expected to modify blood flow and, in the case of breast cancer, should translate into changes in skin surface temperature.

Recently, the availability of digital infrared imaging devices has allowed a reassessment of infrared imaging as a screen for breast cancer, particularly in combination with mammography, with promising results being obtained.^{2,3} Furthermore, dynamic infrared imaging, which measures skin temperature changes resulting from rapid oscillations in skin blood perfusion, also allows objective discrimination between benign and malignant tumours.⁸ A typical infrared image of a breast tumour reveals a 1–2°C elevation in skin surface temperature at the periphery of the tumour, with the tumour mass often being associated with a corresponding reduction in skin surface temperature. The cause of these temperature changes is unclear, but it has been suggested that the elevation in

temperature at the tumour periphery is due to hypervascularity around the tumour as a result of tumour-induced angiogenesis. Surprisingly, infrared imaging has been rarely used to monitor tumour growth in experimental animals. Of particular interest was whether similar tumour-associated changes in surface temperature occur in animals as in humans and whether such changes can be used to monitor tumour growth and angiogenesis. If infrared imaging can be used as a noninvasive procedure for assessing tumour angiogenesis, it could be employed as a simple method for monitoring the activity of antiangiogenic drugs in animals and in cancer patients.

MATERIAL AND METHODS

Infrared imaging of tumours

All animal experimental protocols used in our study were approved by the Australian National University Animal Experimentation Ethics Committee and were carried out according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The highly metastatic rat mammary adenocarcinoma 13762 MAT was used in all experiments, the tumour cells being maintained *in vitro* as previously reported.⁹ In the primary tumour growth assays, immunocompetent female Fischer 344 rats (10–13 weeks of age) ($n = 5$) were injected subcutaneously into the hind midabdomen with 10^6 13762 MAT tumour cells in 100 μ l RPMI 1640 medium (Gibco BRL, Grand Island, NY) containing 10% FCS. Control animals ($n = 3$) were injected with FCS containing medium without tumour cells. The hind midabdomen was chosen as the injection site as it was easy to position the animals for scanning and this region has a relatively uniform normal skin surface temperature range. In a room with constant temperature, rats were anaesthetised with ether at various time points and whole body digital infrared images collected using a Compix PC2000 Thermal Imaging System (Compix Inc., Tualatin, OR). This infrared camera contains a single element lead selenide detector with a waveband of 3–5 μ m and has a thermal resolution of 0.1°C, a spatial resolution of 0.4 mm at the distance (15 cm) used for imaging and an image capture rate of 15 sec. At the same time, if appropriate, tumour diameters were measured in mm by dial callipers. To serve as controls, digital infrared images were

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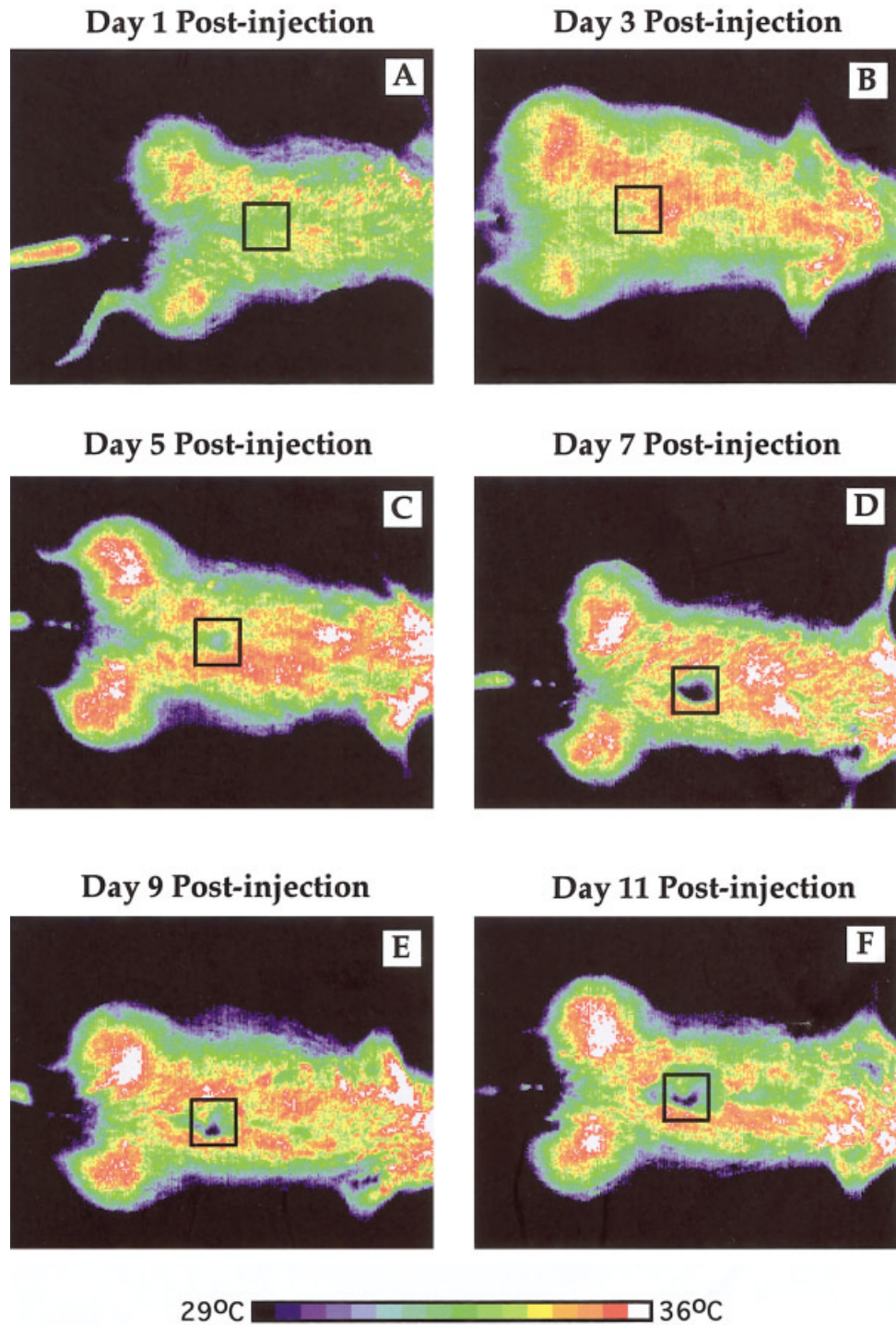


FIGURE 1 – Whole body digital infrared images of the same rat at various times after implantation of the rat mammary adenocarcinoma 13762 MAT. The box in each image (approximately 2 cm × 2 cm) corresponds to the area of tumour growth, with blue-green indicating a reduction in skin surface temperature and yellow-red-white an increase in temperature (see scale bar).

collected immediately before tumour cell injection and immediately after injection of the tumour cells. The latter image served as a record of the tumour cell injection site, as the injected medium was at room temperature and therefore produced a transient reduction in skin surface temperature at the site of injection.

Data analysis

Digital infrared images were analysed using Compix Thermal Evaluation software (TES Version 1.210). Surface temperature plots were generated using NIH Image software (Version 1.62,

National Institutes of Health, Bethesda, MD). The range in surface temperature for an approximately 2 cm × 2 cm square, which corresponded to the site of tumour growth, was determined for each animal, with all pixels in the square being used for calculations. The effect of the presence of a tumour in each animal on the surface temperature range was calculated according to the formula: tumour-induced surface temperature change (°C) = temperature range of tumour area – mean temperature range of same area in each animal before tumour cell injection. Such a calculation eliminated any day-to-day and animal-to-animal variations in the actual

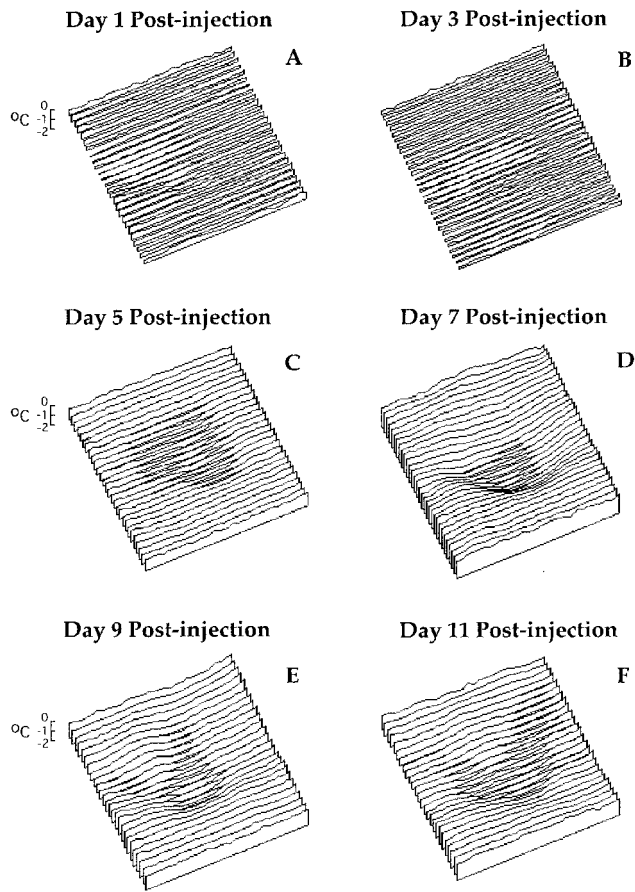


FIGURE 2 – Surface temperature plots of areas of tumour growth (boxed areas in Fig. 1) at various times after 13762 MAT tumour cell implantation.

skin surface temperatures. The temperature range represented the coldest and hottest point for each scan, with one value for surface temperature change being calculated for each animal.

As a control, a similar calculation was performed on animals that were injected with medium rather than tumour cells. The values were analysed by a 2-tailed unpaired Student's *t*-test between the tumour-bearing group and the medium-injected control group. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Previous studies from our group have used the highly invasive rat mammary adenocarcinoma, 13762 MAT, as a tumour model for screening for novel antimetastatic and antiangiogenic compounds.¹⁰ In fact, the antiangiogenic drug PI-88, which is currently undergoing clinical trials in cancer patients, was initially identified using the 13762 MAT model.¹⁰ Thus, the 13762 MAT mammary adenocarcinoma represents an excellent animal model for examining the effects of subcutaneous tumour growth on skin surface temperature and whether angiogenesis-induced changes in vascular density plays a role in any surface temperature changes observed.

Figure 1 presents infrared images generated from a single representative animal at various times after subcutaneous injection of 13762 MAT tumour cells. At days 1 and 3 after tumour cell administration, no discernible reduction in skin surface temperature was observed (Fig. 1*a* and 1*b*), but at days 5, 7, 9 and 11 after injection (Fig. 1*c-f*), a clear reduction in surface temperature at the

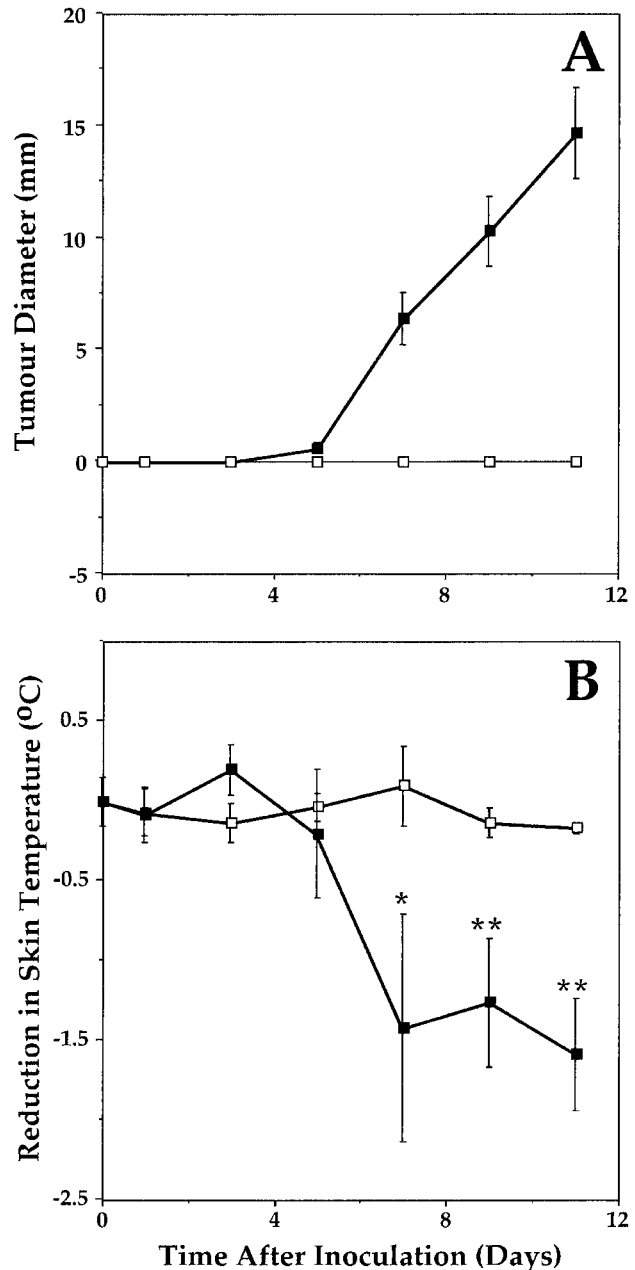


FIGURE 3 – Relationship between tumour size (panel *a*) and reduction in tumour-associated skin surface temperature (panel *b*) in rats at various times after implantation of the rat mammary adenocarcinoma 13762 MAT. Data for control animals (□) (*n* = 3) and animals receiving 13762 MAT tumour cells (■) (*n* = 5) are depicted. Vertical bars represent the standard error of the mean (SEM) with significant differences between control and tumour bearing animals in panel *b* indicated by asterisks (**p* < 0.05, ***p* < 0.001). Results are representative of 3 separate experiments.

site of tumour growth was detected. A palpable tumour was first detected on day 5 and continued to grow at later time points. Surface temperature plots of the area of tumour growth (boxed areas in Fig. 1) are presented in Figure 2 and highlight the reduction in skin surface temperature at days 5, 7, 9 and 11. Before a palpable tumour appeared (days 1 and 3) there was little variation in the skin surface temperature (< ± 0.5°C), but once a palpable tumour appeared, a clear area of reduced temperature (> 1°C) was

evident. An important feature of the temperature data depicted in Figures 1 and 2 is that there is no evidence of an elevation in skin surface temperature at or adjacent to the site of tumour growth, unlike that reported in breast cancer patients.^{2,3} Only a reduction in skin surface temperature can be seen. This observation was consistently made in all tumour-bearing animals examined, although the animal depicted in Figures 1 and 2 had a 2-day earlier onset of tumour growth than most animals examined. Thus, when several tumour-bearing animals were assessed, at days 7–11 after tumour cell injection there was a constant 1.5°C reduction in skin surface temperature, a change that was not observed in a control group of animals that did not receive tumour cells (Fig. 3). This temperature change was highly significant (p value < 0.001 on days 9 and 11), was independent of tumour size and was usually observed with tumours ≥ 0.5 cm in diameter (Fig. 3). Furthermore, the surface temperature plots in Figure 2 indicate that the area of reduction in skin surface temperature is independent of tumour size, the area of temperature reduction remaining remarkably constant on days 5–11 (compare Fig. 2c–f).

DISCUSSION

With the emergence of angiogenesis inhibition as a viable approach to cancer therapy,^{4–7,11,12} there has been considerable interest in the development of simple, noninvasive procedures that can monitor the *in vivo* activity of new antiangiogenic drugs. Because it has been proposed that angiogenesis-induced hypervascularity around breast tumours may produce elevated skin surface temperatures that can be readily measured by infrared imaging,^{2,3} this proposition was investigated in tumour-bearing animals. Surprisingly, unlike breast cancer patients, no elevation in skin surface temperature was observed in any tumour-bearing animals despite the well vascularised and highly invasive rat mammary adenocarcinoma 13762 MAT being used in our study. In fact, a constant reduction in skin surface temperature was observed that was independent of tumour size but was produced by relatively small tumours (≥ 0.5 cm in diameter).

Clinical studies in breast cancer patients suggest that a reduction in skin surface temperature can sometimes be associated with the tumour mass, but a considerable elevation in surface temperature is often observed at the periphery of the tumour.^{2,3} Thus, it appears that our animal model is only recapitulating one aspect of the clinical picture. One possible explanation for this discrepancy is that we are dealing with a rapidly growing transplantable tumour, whereas breast tumours in patients have evolved *in situ* over a considerable period. It should be noted, however, that there is considerable angiogenic activity associated with the 13762 MAT tumours, implying that other mechanisms may be inducing elevations in surface temperature at the periphery of tumours. In fact, despite angiogenesis being induced by tumours, the new vessels generated are often not functional,^{4–7} and consequently most solid tumours exhibit hypovascularity and have a reduced blood flow compared to healthy tissues. Such a phenomenon would be expected to result in a reduction in skin surface temperatures, a possible explanation for the results reported here. In this regard, an

earlier study with human tumours transplanted into immunodeficient athymic nude mice reported that all 137 tumours scanned were hypo- or isothermic, with no elevation in skin surface temperature being observed.¹³ Although general hypovascularity of the tumours may explain this effect,^{4–7} an alternative possibility is that subcutaneous tumour growth is stimulating the autonomic nervous system to induce vasoconstriction at the skin surface.⁸

Another possible explanation for the tumour-associated reduction in skin surface temperature observed in our study is that the ether anaesthesia, used to immobilise the animals, is perturbing skin temperature regulation. This explanation seems unlikely, however, as when pentobarbital anaesthesia was used similar surface temperature changes were obtained with human tumours transplanted into nude mice.¹³ Also, pentobarbital anaesthesia has been successfully used to immobilise animals whilst monitoring surface temperature elevation during neurogenic inflammatory responses.¹⁴ In addition, in the absence of anaesthetics, an early study using a thermistor probe to quantify the skin surface temperature of tumours in mice showed a similar 1–2°C reduction in the temperature of the skin covering the tumours.¹⁵

In contrast, it is conceivable that the elevation in skin surface temperature reported in breast cancer patients, but not observed in our study, is associated with a chronic inflammatory response around the developing breast tumours. Unlike *in situ* tumour development, which is characteristic of cancer patients, chronic inflammation would be expected to not normally occur with rapidly growing transplantable tumours such as 13762 MAT. A hallmark of inflammation is vasodilation with a resultant increase in blood flow and skin temperature. Indeed, infrared imaging has been successfully used to monitor inflammation in rheumatic diseases.¹⁶ It has also been suggested that the hyperthermia associated with breast tumours is due to nitric oxide-mediated vasodilation,¹⁷ with local nitric oxide production being a feature of many inflammatory responses.¹⁴

An interesting feature of inflammation is that it appears to be associated with a poor prognosis in cancer patients. Thus, tumour infiltrating macrophages are often proangiogenic,^{18,19} favour tumour growth^{18,19} and have been associated with a poor prognosis in melanoma and breast cancer patients.^{19,20} Based on this hypothesis, it could be argued that infrared imaging may identify breast tumours that are inducing a strong inflammatory response, such a response being likely to be associated with a poor prognosis. Possible support for this suggestion comes from the report that breast cancer patients with “abnormal thermograms” have tumours that express growth rate related markers suggestive of faster growing and more highly metastatic tumours.²¹ Clearly, additional clinical and animal studies are required to verify this important concept.

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