Short-term effects of axillary lymph node clearance surgery on lymphatic physiology of the arm in breast cancer

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BREAST CANCER-RELATED LYMPHEDEMA (BCRL) is a distressing condition caused by surgery and/or radiotherapy to the axilla in patients with breast cancer. Although it is clear that surgical removal of lymph nodes from the axilla is the single most important event in BCRL, the pathophysiological mechanisms that cause edema are poorly understood (24) and would need to account for a number of clinical observations, such as an onset that is often delayed for months or even years, and the phenomenon of “sparing,” in which parts of an otherwise swollen arm (often the hand) remain unaffected. Several pathophysiological observations are also unexplained, such as the finding of a protein concentration in the interstitial fluid of the epifascia of the swollen arm that is lower than would be expected in edema of reduced lymph flow (2).

The majority of women (~75%) undergoing axillary lymph node resection never develop BCRL (15). Although the compensatory protective mechanisms are unknown, it has been suggested that they include the opening up of anatomical lymphovenous communications in the arm (1), rerouting of lymph through lympholymphatic communications distal to excised nodes (4, 9) and increased macrophage-mediated tissue proteolysis, allowing the escape of low-molecular weight peptides and protein fragments via the blood (5, 6).

Most studies of the effects of axillary lymph node clearance surgery have been on patients with established BCRL. Early pathophysiological responses to surgery, however, may provide important clues as to the cause of the condition. The aim of the present study, therefore, was to investigate the effects of surgery on the transport or mobilization of extravascular protein into local blood of the operated, ipsilateral arm as well as into central blood.

METHODS

Subjects

The study population consisted of 16 women with recently diagnosed breast cancer ranging in age from 39 to 76 yr (mean 58 yr) (Table 1). Each patient was studied before and after axillary lymph node clearance surgery. The period between the first study and surgery ranged from 1 to 31 days (mean 12 days) and between surgery and the second study from 70 to 119 days (mean 90 days). Of the 16 patients, 11 had mastectomy, whereas 5 had wide local excision. None developed any infection, but one patient had a seroma. Five patients received radiotherapy to the breast and seven to the chest wall (of whom, 3 also had radiotherapy to the supraclavicular region). Ten patients received tamoxifen, two neoadjuvant chemotherapy, and four adjuvant chemotherapy. By the time of 3-yr follow up, four patients had developed clinical evidence of BCRL, defined as an arm volume of 10% or greater compared with the contralateral arm (allowing for preoperative differences).

Radiolabeled polyclonal human immunoglobulin G (HigG) was used as a tracer for protein transport because, unlike human serum albumin, it can be labeled with good stability with 111In or 99mTc and used as a tracer for protein transport because, unlike human serum albumin, it can be labeled with good stability with 111In or 99mTc and is therefore well suited to dual isotope studies. Thus the rates of depot clearance and accumulation in central blood of 111In-HigG on the other, before and 3 mo after axillary clearance surgery. The rates of clearance of activity from the depot (k) and accumulation in central blood (b contra) were measured using a scintillation probe and bilateral antecubital vein blood sampling, respectively. Activity accumulating in blood ipsilateral to the injected side, in excess of central blood activity (b ipsi) was also calculated as a measure of local vascular uptake. The k correlated with b contra, but neither changed in response to surgery. However, b ipsi for injections of 99mTc-HigG into the affected arm increased in all seven patients in whom data were available (0.018 ± 0.006 to 0.038 ± 0.007%/min; P < 0.05); indeed, in five of these seven, b ipsi paradoxically exceeded b contra, and none developed BCRL at 3-yr follow-up. We conclude that uptake of protein into local blood and/or proteolysis increases after axillary surgery and may protect against BCRL.

99mTc-immunoglobulin; proteolysis

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Table 1. Study population

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Y, yes; N, no; BCRL, breast cancer-related lymphedema.

Data Analysis

Depot clearance rate. The counts recorded at each time point at the depot of injected radiolabeled HlgG were fitted with a single exponential, and the rate constant (k) was calculated. The value of k is dependent on several processes, including diffusion in the interstitial fluid away from the depot and possible direct access to local blood capillaries, but is generally assumed to be largely dependent on local lymph flow (13), quantification of which is routinely based on k in clinical lymphoscintigraphy (26).

Blood radiolabeled HlgG recovery. Blood concentrations of radio-labeled HlgG, sampled from the antecubital vein contralateral to the side of injection, were recorded as percent of administered activity per liter of blood. The total amount of circulating radioactivity, expressed as a percentage of administered activity, was obtained by multiplication of the blood concentration in each sample by the subject’s blood volume in liters, estimated from height, weight, gender, and age using a standard conversion equation (20). The total blood accumulation rate (bcontra), as determined from contralateral sampling, was essentially linear and fitted by linear regression analysis to give a slope with units of percent of administered dose per minute.

Blood concentrations of radiolabeled HlgG, sampled from the ipsilateral antecubital vein, were recorded as percent of administered activity per liter of blood. The contralateral time-concentration curve was subtracted from the ipsilateral curve to record a curve that is corrected for recirculating activity. Using the principle of indicator dilution (i.e., principle of conservation of mass as in the Stewart-Hamilton equation for measurement of blood flow), the recirculation-corrected ipsilateral time-concentration curve was then integrated over 3 h (estimating concentrations at 135 and 165 min by interpolation) and compared with an assumed value for the local forearm blood flow that contributed to the dilution of radioprotein to obtain an estimate for total amount of radioactivity (M) accumulating in local ipsilateral blood as a function of time (18):

\[ M(t) = \text{blood flow} \times \text{area under ipsilateral time-concentration curve}(t) \]

where \( t \) is time. Effective forearm blood flow, which is not necessarily the same as total forearm blood flow, was conservatively assumed to be 20 ml/min, based on a forearm volume estimate of 1 liter and a forearm blood perfusion of 2 ml·min⁻¹·100 g⁻¹ (22). As for contralateral sampling, the profile of radioactivity accumulating ipsilaterally was fitted by linear regression analysis to give the rate of ipsilateral protein accumulation (bipsi) in units of percent of administered activity per minute.

It has previously been shown in normal subjects that \(^{111}\text{In}-\text{HlgG}\) and \(^{99m}\text{Tc}-\text{HlgG}\) give the same values of k and bcontra. Differences between the two tracers, however, were recorded from ipsilateral blood sampling (18). This was almost certainly because ipsilateral sampling is highly sensitive to small amounts of protein-free tracer; radioactivity associated with solutes of small molecular size preferentially enter local blood vessels instead of lymphatics, and this was more of a problem with \(^{111}\text{In}-\text{HlgG}\) than with \(^{99m}\text{Tc}-\text{HlgG}\). The bipsi based on \(^{111}\text{In}-\text{HlgG}\) was not therefore included for analysis.

Tissue-to-blood transport. The amount of radioactivity that had accumulated in central blood (as measured by contralateral sampling) and in the local vasculature (as measured by ipsilateral sampling and restricted to \(^{99m}\text{Tc}\) at 3 h was divided by the amount of \(^{99m}\text{Tc}\) that had left the depot at the same time to give central and local tissue-to-blood (TB) transport, respectively, as previously described (17, 18).

Statistical Analysis

All variables described in Data Analysis were expressed as means ± SE. Associations between variables were quantified using Pearson’s correlation coefficient. A P value of <.5% was regarded as statistically significant.

RESULTS

Depot Clearance

There was no difference between k, respectively, based on \(^{111}\text{In}\) and \(^{99m}\text{Tc}\) in normal, contralateral arms, so the data based on the separate radionuclides were pooled. Mean k in the affected arm was 0.12 ± 0.007%/min, not significantly different from the mean value after surgery, which was also 0.12 ± 0.014%/min, although there was a wide range of changes (post/pre: 0.36–2.3). There was less variation in k before than after surgery, with respective coefficients of variation (standard deviation divided by mean) of 24 and 44% (Fig. 1).
Contralateral Blood Recovery

As for \( k \), there was no difference between \( b_{\text{contra}} \), respectively, based on \(^{111}\text{In} \) and \(^{99m}\text{Tc} \) in normal, contralateral arms, so the data based on the separate radionuclides were pooled. Mean \( b_{\text{contra}} \) in the affected arm was \( 0.068 \pm 0.009\%/\text{min} \), which was not significantly different from the mean value after surgery, which was \( 0.053 \pm 0.0.011\%/\text{min} \). As with \( k \), there was a wide range of changes (post/pre: 0.15–2.2) and more variation after surgery, with respective coefficients of variation before and after of 49 and 73\%, respectively (Fig. 2). There was a significant correlation in \( b_{\text{contra}} \) between the two arms before surgery (\( r = 0.72, P < 0.01 \)) but not after.

The \( k \) correlated significantly with \( b_{\text{contra}} \) in both arms before surgery \( (r = 0.57 \ (P < 0.05) \) and \( 0.75 \ (P < 0.01) \), affected and unaffected, respectively \) and in both arms after surgery \( (r = 0.96 \ (P < 0.001) \) and \( 0.62 \ (P < 0.05) \), affected and unaffected, respectively \) (Fig. 3). The fractional pre- to postoperative change in \( k \) in the affected arm also correlated with the fractional change in \( b_{\text{contra}} \) (Fig. 3, inset).

Central TB Transport

There was no difference between central TB transport based on \(^{111}\text{In} \) and central TB transport based on \(^{99m}\text{Tc} \) in normal, contralateral arms, so the data based on the separate radionuclides were pooled. Before surgery, central TB transport was \( 0.56 \pm 0.049 \), not significantly different from the postsurgical mean value, which was \( 0.49 \pm 0.043 \). As with \( b_{\text{contra}} \), there was a significant side-to-side association before (\( r = 0.68, P < 0.001 \)) but not after surgery.

Ipsilateral Blood Recovery

Ipsilateral blood \(^{99m}\text{Tc} \) concentration was higher postoperatively than preoperatively in all patients at all time points (Fig. 4). Accordingly, \( b_{\text{ipsi}} \) was higher after surgery in all seven patients in whom it could be measured, increasing from a mean preoperative value of \( 0.018 \pm 0.006 \) to \( 0.038 \pm 0.007\%/\text{min} \) after surgery (\( P < 0.05 \)). Cumulative ipsilateral activity expressed as a quotient of contralateral activity also increased after surgery in all seven patients so prominently that to facilitate comparison the ratio was log transformed (Fig. 5). Indeed in five of the seven subjects, cumulative ipsilateral activity exceeded contralateral activity, a finding that was not recorded in any patient preoperatively, in any of the patients in whom \(^{99m}\text{Tc} \)-\text{HlgG} was injected into the unaffected arm or in any of our previously studied normal controls.

Fig. 1. Human IgG (HlgG) depot clearance from the affected upper limb before (○) and after (●) axillary lymph node clearance surgery \((^{99m}\text{Tc} \) and \(^{111}\text{In} \) combined), expressed as the percentage of the initial count rate. Values are mean ± SE of all patients for each time point. Note the wider SE values (vertical bars) after surgery.

Fig. 2. Central blood accumulation recorded after \(^{99m}\text{Tc} \)-HlgG injection into the affected upper limb before (○) and after (●) axillary lymph node clearance surgery \((^{99m}\text{Tc} \) and \(^{111}\text{In} \) combined), expressed as the percentage of the administered activity. Values are means ± SE of all patients for each time point.

Fig. 3. Relation between the rate constant (\( k \)) and total blood accumulation rate (\( b_{\text{contra}} \)) in ipsilateral (circles) and contralateral (triangles) limbs before (open symbols) and after (closed symbols) surgery \((^{99m}\text{Tc} \) and \(^{111}\text{In} \) combined). Inset: relation between the corresponding fractional changes in \( k \) and \( b_{\text{contra}} \) resulting from surgery.
Local TB Transport

Local TB transport increased from a mean preoperative value of 0.17 ± 0.046 to a mean postoperative value of 0.41 ± 0.083 (n = 7; P < 0.02; Fig. 6).

Correlations Between Ipsilateral and Contralateral Blood Recoveries

When values for cumulative ipsilateral activity were compared with corresponding contralateral activities up to all individual blood sampling time points before surgery, strong positive correlations were seen at early time points (strongest at 30 min) with a slight decrease in the coefficient at later time points (Fig. 7A). Surgery, however, completely abolished these correlations and in fact reversed them all to negative correlations, although, unlike the presurgical correlations, they were not significant.

Correlations Between Preoperative and Postoperative Blood Recoveries

Cumulative ipsilateral activities up to individual time points before surgery correlated significantly with the postoperative values at the corresponding times (Fig. 7B). The correlations, however, between correspondingly timed contralateral activities before and after surgery were all weak and negative.

Relation Between Lymphatic Function and BCRL

There was no clear association between the development of BCRL and any of the pre- or postoperative indexes of lymphatic function, either on the affected or unaffected side. It is noteworthy, however, that none of the seven patients in whom there was increases in b\textsubscript{ipsi} and local TB transport developed BCRL.

DISCUSSION

It is not known why only a minority of women undergoing axillary lymph node clearance surgery develop BCRL (24). Those who do not develop BCRL presumably acquire alternative pathways for protein removal from the arm that may be anatomical [such as rerouting of lymph through lympholymphatic or lymphovenous (1) communications distal to excised nodes (4, 9)] and/or functional (such as increased protein transport directly into local blood vessels). The present study examines the second of these two general mechanisms.

Protein is normally transported in lymph from the interstitial space of the arm to central blood via the lymphovenous communications in the neck. It is believed that microvascular fluid flows from capillary lumen to interstitial space, but not in the reverse direction, even into venules (3). According to this
Fig. 7. A: correlation coefficients between the amounts of $^{99m}$Tc that had respectively accumulated in ipsilateral and central blood are shown for all sampling time points after injection into the affected upper limb before (●) and after (▲) axillary lymph node clearance surgery. Before surgery, the correlations were all positive and mostly statistically significant, but after surgery, in contrast, they were all negative, although, individually, not significantly. Insets: relations recorded at 30 min, significant preoperatively but not postoperatively.

B: correlation coefficient between the amounts of $^{99m}$Tc that had accumulated respectively before and after axillary lymph node clearance surgery is shown for all corresponding sampling time points after injection into the affected upper limb. ○, Accumulation in ipsilateral; ▲, accumulation in central blood. Correlations were all positive for ipsilateral accumulation (several statistically significantly) but in contrast were all negative (but not significantly) with respect to accumulation in central blood. Insets: relations recorded at 30 min, significant preoperatively but not postoperatively.
belief, lymph flow must be equal to capillary fluid filtration. Because it is also believed that macromolecular transport from blood capillary lumen to interstitial space is overwhelmingly through convection, there is, under normal circumstances, no significant protein transport through the capillary from interstitial space to blood (14). If after surgery, however, protein was cleared from the extravascular space by transport into local blood vessels, it could be achieved either via peripheral anatomical lymphovenous communications that open up after surgery or directly across the blood capillary endothelium, possibly mediated through an increase in interstitial pressure resulting from surgery. Moreover, this could be facilitated by enzymatic degradation of protein into fragments with higher diffusibility (5, 6).

There is evidence of lymphovenous communications in both humans (1) and experimental animals (8, 7, 21). There is also evidence that protein can transfer directly across endothelium from interstitial fluid to capillary lumen, even physiologically (11, 12, 22), and indeed significant radioactivity was detected in ipsilateral blood preoperatively in the current patients. Although we cannot confirm that it was protein bound in the current patients, and although ipsilateral sampling is heavily influenced by even small levels of protein-free activity, our laboratory has previously shown that a significant proportion of the activity in ipsilateral blood in normal subjects is protein bound (17, 18), especially with respect to 99mTc-HlgG. The present results are therefore particularly interesting because they suggest that, after surgery, protein appears to be diverted toward direct local vascular access. Although the mechanism is not clear, this would clearly tend to offer protection against edema. Indeed none of the seven patients in whom ipsilateral 99mTc activity increased developed BCRL, as opposed to four for the entire population (25%)

The possibility needs to be considered that ipsilateral radioactivity concentration was increased not as a result of increased transport of intact or degraded radioprotein but as a result of a reduction in the blood flow diluting the radioactivity that leaves the depot to enter blood vessels. This blood flow is not identical to forearm blood flow but is presumably closely related to it. This explanation, however, seems highly unlikely from earlier work based on Doppler ultrasound showing that upper limb blood flow increases after axillary lymph node clearance both early and late after surgery but with an overall doubling of activity after surgery support the view that local protein transport from interstitial space to blood does take place. We have previously shown that, when there is substantial protein-free activity in ipsilateral blood, the correlation between cumulative ipsilateral and contralateral blood contents tends to be negative (18), presumably because free activity is cleared rapidly from the central circulation once it arrives there. The inverse correlations observed after surgery suggests either that ipsilateral protein clearance tends to compensate for poor protein transport via lymphatics or that there is local proteolysis.

Whereas the effect of surgery on ipsilateral local protein transport was quite consistent from patient to patient (at least qualitatively), there was a highly variable response with respect to \( k \) and \( b_{cent} \). These two latter variables, however, were closely correlated, making measurement error unlikely as the sole cause of their variability.

In conclusion, we have found a consistent increase in local mobilization of subcutaneously injected protein in response to axillary node resection that might be expected to blunt the tendency to develop BCRL. This increased mobilization is most likely the result of increased local transport from interstitial fluid to blood either directly across the vascular endothelium or through new lymphovenous communications. It may also be due to increased local proteolysis, but the net effect would be the same. Further prospective work is required on larger patient populations to relate this mobilization to the development or otherwise of BCRL and to establish to what extent it is the result of removal of intact protein or increased local proteolysis.

REFERENCES


