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Increased blood flow enhances axon regeneration after spinal transection

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It is not known whether increasing the amount of blood flow to axotomized fibers in mammalian CNS can result in more robust sprouting. To find out, an intact pedicled omentum was surgically transposed to cover a collagen matrix gel used to bridge the transected cat spinal cord stumps. Control animals were similarly treated but did not receive the pedicled omentum. Twelve weeks after cord transection, animals receiving the pedicled omentum showed a 66% spinal cord blood flow increase over animals that did not. Moreover, treatment with the pedicled omentum increased the density of regenerating adrenergic axons 10-fold over the control group. These findings indicate that boosting flow with an omental graft to the collagen bridge site results in robust axonal outgrowth of spinal transected nerve fibers.

A number of hypotheses have evolved within the last 50 years in an attempt to explain why cut mammalian CNS axons do not regenerate effectively. Limiting factors are presumed to be: (a) an unfavorable or hostile tissue terrain, (b) absence of trophic or guiding factors, (c) presence of chemical or physical barriers, and (d) insufficient blood flow [5]. Although bridge grafts of various materials have been tested in spinal cord-transected models, significant axonal regeneration across the implant continues to be a problem [10, 11, 12]. The presence of adequate blood flow is recognized by most investigators to be a critical factor in the recovery of CNS homeostasis after trauma. There is no general agreement, however, as to what constitutes 'adequate' blood flow following CNS axotomy. Moreover, the concept that vigorous spinal axon regeneration after axotomy may be blood flow dependent has not been explored [5].

To test this concept, two questions were addressed:

(a) Can a pedicled omentum surgically grafted to cover a collagen bridge implant after spinal transection substantially increase local tissue blood flow?

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(b) If local tissue blood flow is increased, will density and/or rate of axonal outgrowth near the bridge implant be affected?

To construct this experiment, we combined two surgical techniques using a cat spinal cord transection model: first, a collagen matrix (CM) gel was used to bridge the transected cord stumps; second, an intact pedicled omentum was transposed to cover both collagen matrix and part of the proximal–distal cord stump regions. We have reported elsewhere the ability of collagen matrix to provide a bridge for axonal regeneration on a spinal transected rodent model [4], and the potential of increasing vascular density and blood flow to damaged feline spinal cord using a pedicled omentum [10]. These findings have been confirmed by others [1, 6].

Fourteen female cats were intubated and anesthetized with penthrane/oxygen. A sterile laminectomy exposed T_{8-10}. The dura was opened circumferentially at the transection site and the cord was cleanly transected at T_9 using a very fine-edge blade. A gap of 6 mm was consistently obtained following total cord transection. Total cord separation was verified microscopically by gently reflecting the two stumps. The stumps were irrigated with ice-cold saline until bleeding and CSF leakage had stopped at which time the gap area was dried with cotton wicks. Animals were randomly separated into 3 treatment groups of 4 cats per group and the gap between cord stumps was filled with: (a) collagen matrix (COL group), (b) collagen matrix + pedicled omentum (COM group), (c) gelfoam (GEF group). CM at 4°C was dispensed sterile from a tuberculin syringe and required about 1 h at body temperature to harden into a gel [4]. CM has been shown to be biocompatible, bioabsorbable and capable of creating a tight junction with host CNS tissue [4]. In the COM group, a small laparotomy incision was made, the pedicled omentum was gently removed from the abdominal cavity, surgically lengthened [9] and tunneled subcutaneously to the dorsal cord region where it was secured to the surface of the CM bridge.

All incisions were closed in layers; animals were allowed to recover and postsurgical care was provided for 90 days. On the day of sacrifice, serial spinal cord blood flows (SCBF) were measured in all groups using the hydrogen clearance technique. SCBF was determined by the initial slope method [5]. Ag/AgCl reference electrode was placed in a subcutaneous pouch and Teflon-coated, platinum–iridium microelectrodes were inserted at the center of the CM bridge in COM and COL groups and center of tissue regenerate in GEF animals. Microelectrodes were also inserted in the cord gray matter 12 mm *proximal* and 6 mm *distal* to the center of the original transection site. After 4 blood flows per spinal region had been recorded in all groups, the *distal* cord microelectrodes were left in place in the COM group and the pedicled omentum was occluded by ligation several centimeters from its junction with the CM. SCBF measurements were repeated until fluctuating values had stabilized for 15 min (Fig. 1).

Animals underwent cardiac fixation perfusion, the cord tissue was removed, blocked and sectioned 16 μm longitudinally in a cryostat. After incubation with the appropriate antibody, sections were processed for peroxidase–antiperoxidase and immunofluorescence to tyrosine hydroxylase-immunoreactive axons (TH-IR). Alternate longitudinal sections were taken for Palmgren's silver axon and Nissl stains. A

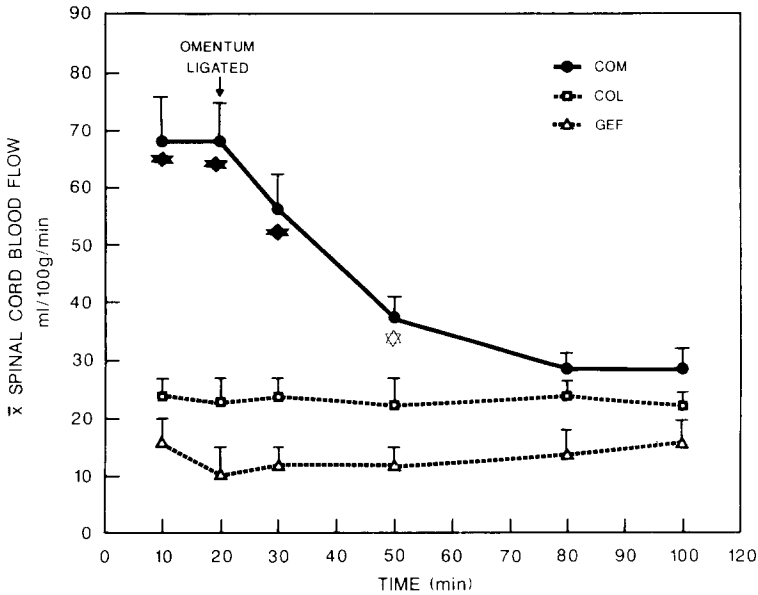

Fig. 1. Mean averages of 6 serial spinal cord blood flow (SCBF) measurements in groups COM (collagen matrix + omentum), COL (collagen matrix) and GEF (gelfoam). Figure shows flows recorded 6 mm distal to the transection site on day 90 after surgery. Note rapid linear fall of blood flow in COM group from 68 ml/100 g/min to a mean 28 ml within 1 h following pedicled omentum ligation (arrow) in COM-treated animals. Mean SCBF in COM was compared with COL and GEF groups at each sampling time point using analysis of variance (ANOVA); closed star: $P<0.0001$, open star: $P<0.001$. Hydrogen clearance/microelectrode technique [4]. Vertical bars are standard error of the mean.

25 microsquare eyepiece grid comprising a 625 μm^2 area was used on every 10th section to count and average the number of blood vessels and TH-IR axons/dry high power field (HPF) within the CM and distal cord tissue at 1 mm intervals.

SCBF measurements recorded 12 mm proximal to the transection site were not significantly different among the experimental 3 groups or intact controls, with values ranging from 54 to 71 ml/100 g/min. Blood flows in the mid-CM region (COL, COM groups) or center of tissue growth (GEF group), averaged 37 ml ($\pm$8 ml) in COM, 18 ml ($\pm$5 ml) in COL and 7 ml ($\pm$2 ml) in GEF group. Blood flows recorded 6 mm distal from the transection site, averaged 68 ml ($\pm$12 ml) in COM, 24 ml ($\pm$6 ml) in COL and 12 ml ($\pm$3 ml) in GEF-treated cats (Fig. 1). Mid-collagen and distal cord SCBF differences between COM and COL or GEF group were statistically significant (Fig. 1).

Within a few minutes following ligation of the pedicled omentum in the COM group, a time-related linear fall in distal cord blood flow was observed which levelled off at about 28 ml after 1 h post occlusion (Fig. 1). The increased preligation blood flow values observed in the COM group were assumed to be the omentum's contribution to the distal cord and represented an 83% difference when compared to GEF group blood flows and a 65% difference from COL treated animals (Fig. 1). Blood

vessel density counts of the CM bridge showed a 3:1 higher ratio (average 5.6 vessels/HPF) for COM as compared to COL (average 1.8 vessel/HPF) treated animals, a finding that supported the relative increase in SCBF measured in the COM group at the end of the survival period. Blood vessel density at the center of tissue growth in GEF group was too low for comparative analysis.

When counts of TH-IR fibers within the CM bridge and distal cord were averaged, a 10- and 8-fold increase respectively was observed in COM over COL treatments. Fig. 2a,b shows typical TH-IR axon density in COL (average 2.1 axons/HPF) and COM (average 23.4 axons/HPF) treated animals respectively at the mid-CM bridge level. No TH-IR axons were seen within the tissue regenerate or distal to it in GEF animals.

It is known that tyrosine hydroxylase is the biosynthetic enzyme for catecholamines and that descending aminergic tracts in spinal cord originate from neurons located in the brain [3, 5, 13]. The presence of TH-IR fibers below the site of transection in the COM group suggests that these axons sprouted from disconnected supraspinal adrenergic neurons. Retrograde axon tracing studies however, are needed to establish unequivocally the specific neuronal origin of these TH-IR regenerated axons. Moreover, additional outgrowth from non-TH-IR nerve cells must have also occurred in COM and COL groups because Palmgren sections (a general axonal stain) revealed considerably more silver impregnated than TH-IR fibers near the proximal-CM bridge area. In addition, we have shown in another study using the same parameters as in the present experiment but varying the observation period, that retrogradely labelled neurons are found in the proximal cord of COM treated cats when HRP was injected in the distal cord 48 h before. No labelling of proximal cord neurons was observed in COL or GEF groups [21].

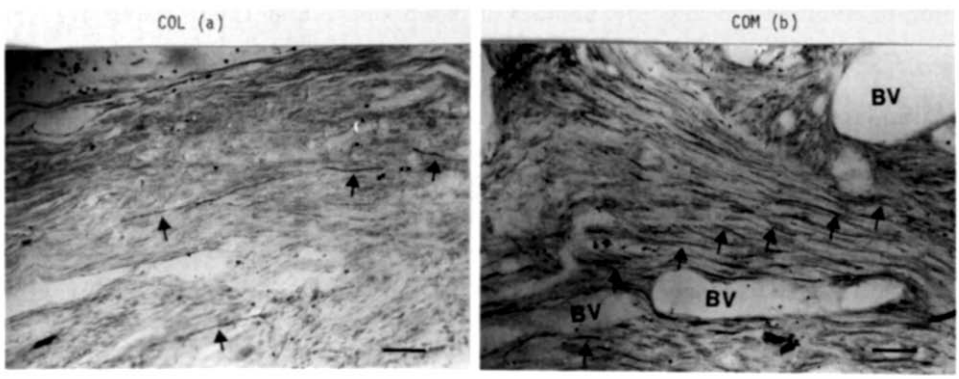

Fig. 2. Tyrosine hydroxylase-immunoreactive (TH-IR) fibers 90 days following transection and implantation of a collagen matrix bridge (COL) or collagen matrix bridge + pedicled omentum (COM). Midpoint in the collagen matrix, a few axons (arrows) are seen in COL group (a); a 10-fold increase in TH-IR fibers (arrows) were counted in the same region of COM-treated animals (b). Many small and large blood vessels (BV) are seen surrounding a robust axonal proliferation in the COM- but not COL-treated cats. Peroxidase–antiperoxidase method [13]. Bar = 50 μm.

These findings indicate that transposing a pedicled omentum to the CM bridge region after cord transection, significantly promotes a greater density and rate of supraspinal-derived regenerating axons presumably as a result of restoring spinal cord blood flow in the host tissue to near normal levels. Three findings support this conclusion:

(1) Significantly higher SCBF was measured in COM than in COL and GEF groups (Fig. 1).

(2) SCBF increase in COM group was reversed when omental blood flow to CM was occluded (Fig. 1).

(3) Marked blood vessel density was found within the CM bridge of COM as compared to COL group (Fig. 2a,b).

Consequently, it appears that improved regeneration seen in the COM group closely correlates with the boosted vascularity obtained from the pedicled omentum graft.

It is possible that in addition to raising the level of nutrients and oxygen resulting from the increased blood flow, other elements could contribute towards the improved axonal regeneration obtained after COM treatment. For example, the omentum may be supplying the sprouting fibers with needed circulating proteins [10], angiogenic [7] or neurotrophic factors [8]. The solution to this problem could provide a fundamental clue in clarifying central axonal regeneration.

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