Spinal cord arteries in *Canis familiaris* and their variations: implications in experimental procedures

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Abstract

To study the normal disposition and variations of the arteries that supply the spinal cord of *Canis familiaris*, a colored solution was injected in the aorta of ten adult mongrel dogs. The dissection of these specimens and the analysis of 2 mm thick diaphanous transversal sections of their spinal cords allowed confirming most of the early reports regarding spinal cord arteries in this species. However, great variability was found in the origin of the ventral spinal artery, in the density of the arteries that form the spinal arterial ring, in the spinal arteries originating from segmental arteries and in the presence of the great ventral medullar artery or Adamkiewicz artery, which was found in only half of the specimens. Comparing the results obtained with those commonly described in humans, it is clear that *Canis familiaris* shows a great similarity in most respects, particularly in those of clinical relevance, namely the greater density of arteries supplying the spinal cord in the cervical and lumbar segments, and the marked variability of the spinal arteries arising from segmental arteries, which end up by playing a major role in the supply of blood caudally to the lower end of the cervical spinal cord. Nevertheless, dogs differ substantially from humans in the origin of the ventral spinal artery and in the origin and presence of the Adamkiewicz artery. Overall, the data show that, from an anatomical standpoint, the dog seems to be a good model for spinal cord ischemia in humans.

Keywords: spinal cord, arteries, *Canis familiaris*, dog, experimental procedures.

1 Introduction

Recently, *Canis familaris* (common dog) species has been extensively used as an experimental model for spinal cord disease. In particular, several papers have been published in the last few years in which the dog was used as a model for spinal cord ischemia (KAPLAN, GRAVENSTEIN, FRIEDMAN et al., 1987; LOWELL, GLOVICZKI, BERGMAN et al., 1992; NAGY, DZSINICH, SELMECI et al., 2002; NAMBU, KAWAHARA, KOBAYASHI et al., 2004; SHIBATA, 2001; SUGAWARA, SUEDA, ORIHASHI et al., 2003; ZHAN, JI, XU et al., 1989). Among these works, dog studies aimed at minimizing the risk of paraplegia after thoracic or thoracoabdominal surgery have received great attention among the scientific community (BERGUER, 1992; ELMORE, GLOVICZKI, HARPER et al., 1992; MARSALA, VANICKY, GALIK et al., 1993; MATSUYAMA, CHIBA, IHAYA et al., 1997; MUTCH, THIESSEN, GIRLING et al., 1995; MUTCH, GRAHAM, HALLIDAY et al., 1993; SIMPSON, EIDE, SCHIFF et al., 1994; TABAYASHI, NIIBORI, KONNO et al., 1993; WILLIAMS, ANDREWS, CHEE et al., 1994; WISSELINK, BECKER, NGUYEN et al., 1994). In addition, there has been a growing interest in the utilization of diverse animal models to validate promising, albeit preliminary, results obtained in the repair of spinal cord injury (LEANNE, LIU, AU et al., 2004). Dogs have been among the animals proposed for these studies (GOLDSMITH, 1994; SMITH, 2002). Therefore, the importance of a thorough knowledge of the arterial supply to the spinal cord of *Canis familiaris* is of indisputable importance.

Although several works have been published concerning spinal cord arteries in dogs (ANDERSON and KUBICEK, 1971; CAULKINS, PURINTON and OLIVER Jr., 1989; GOUAZE, CASTAING and SOUTOUL, 1965; GOUAZ'E, SOUTOUL and CASTAING, 1964; SINDOU, 1979; SOUTOUL, GOUAZÉ, CASTAING et al., 1965; SOUTOUL, GOUAZ'E and CASTAING, 1965; TORRE, MITCHELL, NETSKY et al., 1962; WILSON and LANDRY, 1964), the number of specimens analyzed in each study has usually been small, and not enough attention has been paid to the variability of these arteries. The enormous variability of the arteries that supply the spinal cord has been unmistakably demonstrated in several species, being probably more profusely documented in humans (ALLEYNE, CAWLEY, SHENGELAIA et al., 1998; KAWAHARADA, MORISHITA, HYODOH et al., 2004; KOSHINO, MURAKAMI, MORISHITA et al., 1999; LO, VALLEÉ, SPELLE et al., 2002; MALIKOV, ROSSET, PARASKEVAS et al., 2002; NIJENHUIS, LEINER, CORNIPS et al., 2004).

The objective of this work is to contribute to a more profound knowledge of the arterial supply of the spinal cord in the dog, giving particular attention to the variations found. We will also strive to highlight the implications of our results when planning and executing experimental procedures in this species, and interpreting the data obtained, especially when the aim is to extrapolate the information to humans.

2 Material and methods

Ten adult mongrel dogs were obtained from Lisbon's city kennel. The dogs were sedated with pentobarbital before an injection of a solution containing 10% formol was injected in one of the femoral arteries. Shortly after sacrifice, a median thoracotomy was performed, and the ascending aorta was catheterized close to the brachiocephalic trunk. A small

incision was made in the lateral flank of the cranial vena cava. Then, the aorta was perfused with warm saline (approximately 37 °C) until the blood drained by the severed vena cava was replaced by saline. Next, a solution containing a 60 °C suspension of barium sulphate (Micropaque®-Nicholas Lab.), latex, and red pigment (Super Tintolac®-Robialac) was injected cranially through the catheter inserted in the aorta, according to the technique used in our department (PAIS, 1995). The injection was stoppedwhen the red solution was seen emerging from the cranial vena cava. After the injection was completed, the cranial vena cava and aorta were closed with suture line. The cisterna magna was accessed through a dorsal incision in the cervical region and a solution containing 10% formol was injected into the subarachnoid space, in order to better preserve the central nervous system. The specimen was kept in a refrigerator at a temperature of 4 °C for about 24 hours. Then, the vertebral column and head were removed en bloc, and kept in a solution of formol 10% for a period of at least two weeks, allowing structures to become definitely fixed. Subsequently, all the specimens were carefully dissected under a stereotaxic microscope (Carl Zeiss®), allowing the detailed registry of all the arteries that supplied the spinal cord and their superficial distribution. Finally, the spinal cord was divided into 2 mm thick sections under a microtome and the ordered segments were studied under a binocular magnifying glass (Carl Zeiss®), after being made diaphanous by the technique currently used in our department (PAIS, 1995).

3 Results

The spinal cord receives blood from five longitudinal trunks: the ventral spinal artery (v.s.a) that runs along the ventral median fissure of the spinal cord; and a pair of dorsal spinal arteries (d.s.a) on each side of the dorsal aspect of the spinal cord. Each pair is composed of a larger caliber lateral dorsal spinal artery (l.d.s.a.) and a thinner medial dorsal spinal artery (m.d.s.a.), placed laterally and medially to the dorsal root entry zone, respectively.

The v.s.a. has a variable origin: 20% emerge from the point where the two vertebral arteries (v.a.) converge to originate the basilar artery (b.a.); 20% resulted from the anastomosis of two v.s.a., each coming from the medial flank of the v.a. on the same side; and 60% from only one of the v.a. (4 cases from the right v.a. and 2 cases from the left).

The d.s.a. can originate either from the rostral cerebellar artery (40%) or from the b.a. (60%). For each specimen studied the arteries from both sides had the same origin.

The v.s.a and the d.s.a. extend from their point of origin up to the caudal end of the spinal cord. Near the cauda equina, anastomoses interconnect the v.s.a. and the d.s.a..

Along their course, the v.s.a. and the d.s.a. are profusely anastomosed by horizontal and oblique vessels (Figure 1), forming an irregular arterial network often called spinal arterial ring (s.a.r.). This network is denser in the cervical and lumbar regions in all the specimens studied.

At the level of each intervertebral foramen, the s.a.r. is frequently reinforced by spinal branches of arteries outside the vertebral column (v.a., deep cervical, intercostal and lumbar arteries). Having entered the vertebral canal, each spinal branch gives rise to a ventral and a dorsal radicular artery (v.r.a. and d.r.a., respectively) that accompany the homonymous roots of the spinal nerves to the s.a.r.. The frequency of the individual segmental arteries is depicted in Table 1. We found a spinal artery associated with a spinal nerve in the cervical, thoracic, lumbar, sacral, and coccigeal region with a frequency of 75 ± 14,1%; 78 ± 16, 2%; 69 ± 10,7%; 70 ± 10,0%; 56 ± 5,5% on the right side, and 51 ± 8,3%; 67 ± 11,8%; 64 ± 12, 7%; 67 ± 11,5%; and 62 ± 8,3% on the left side, respectively. On average, 72 ± 14,7% of the spinal nerves on the right side were associated with a spinal artery, and 62 ± 12,0% on the left side.

Statistically analyzing the data concerning the disposition of the segmental arteries by applying the t-student test (BLAND, 2000; UITENBROEK, 1997), we found that:

- globally, spinal arteries were more frequent on the right side ($p < 0,01$); and
- considering each region of the spinal cord in turn, the difference between the number of spinal arteries on the right and left sides was statistically significant only in the cervical and thoracic regions, these arteries being more frequent on the right side ($p < 0,001$ and $p < 0,05$, respectively).

The v.s.a. descends, almost in a straight line, on the ventral surface of the spinal cord within the ventral median fissure up to the lower cervical region. Below that level it tends to become smaller, follows a more sinuous path close to the ventral median fissure and is mostly composed of variable branches originating from the v.r.a., as is shown in Figure 2.

In addition to the relatively small spinal segmental arteries, we found a larger feeder artery, arising from the spinal branch of the fifth lumbar artery, entering the vertebral canal through the intervertebral foramen, and joining the v.s.a. via a characteristic hairpin configuration. However, this artery, often called great ventral medullary artery or Adamkiewicz artery (a.a.), was only found in 50% of the cases studied, originating in all cases from the left fifth lumbar artery. When present, the a.a. supplies the major part of the ventral two thirds of the caudal half of the spinal cord. When it reaches the ventral median fissure, it moves caudally replacing the v.s.a., and it also gives rise, cranially, to an important branch to the thinning v.s.a..

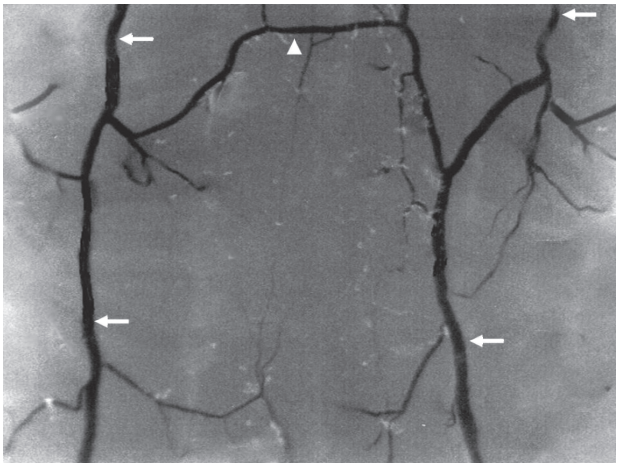

Figure 1. Photograph of a diaphanous coronal section of the spinal cord showing the two medial dorsal spinal arteries (*white arrows*) interconnected via a transverse branch (*arrowhead*), contributing to form the spinal arterial ring.

Table 1. Frequency of segmental spinal arteries at different levels of the spinal cord.

Frequency of spinal arteries (%)		
Level	**Right**	**Left**
C1	50	40
C2	60	50
C3	70	50
C4	80	50
C5	90	70
C6	80	50
C7	90	50
C8	80	50
T1	80	50
T2	80	60
T3	80	70
T4	50	80
T5	100	60
T6	100	90
T7	90	70
T8	70	50
T9	60	60
T10	80	70
T11	70	80
T12	100	60
T13	60	70
L1	60	50
L2	70	50
L3	50	60
L4	80	60
L5	70	70
L6	80	80
L7	70	80
S1	60	60
S2	70	60
S3	80	80
Co1	60	70
Co2	50	60
Co3	50	50
Co4	60	60
Co5	60	70

C - cervical segment of the spinal cord; T - thoracic segment of the spinal cord; L - lumbar segment of the spinal cord; S - sacral segment of the spinal cord; and Co - coccigeal segment of the spinal cord.

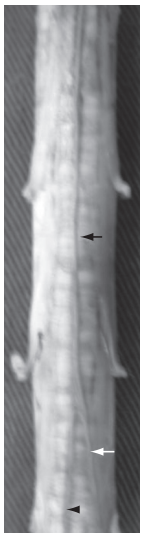

Figure 2. Photograph of the ventral aspect of the lumbar portion of a dog's spinal cord. The *arrows* indicate the ventral spinal artery (v.s.a.); the *arrow heads* point to the ventral radicular arteries (v.r.a.) that accompany the homonymous roots of the spinal nerves, converging into the v.s.a..

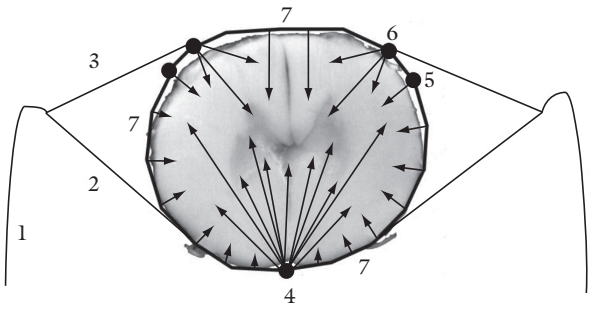

Figure 3. Transverse section of a dog's spinal cord at the level of T4, illustrating the main territories of the arteries that supply the spinal cord in this species. 1 - Spinal branch; 2 - ventral radicular artery; 3 - dorsal radicular artery; 4 - ventral spinal artery; 5 - lateral dorsal spinal artery; 6 - medial dorsal spinal artery; 7 - anastomoses between the longitudinal spinal arteries forming the spinal arterial ring; the *arrows* indicate the pathways followed by spinal perforating arteries emerging from the arteries that form the spinal arterial ring.

The study of diaphanous sections of the spinal cord revealed that the v.s.a. gives rise in its dorsal aspect to many dorsal branches that travel through the ventral median fissure to reach central regions of the spinal cord, namely the ventral horns, the gray comissure, the base of the dorsal horns, the ventral white column, and the ventral two thirds of the lateral white column, as is illustrated in Figure 3. The d.s.a. originate branches that provide the dorsal horns, the dorsal white columns, and the dorsal portion of the lateral white columns. Branches from the irregular network that connects the v.s.a. and the d.s.a. supply adjacent parts of the spinal cord. From the point where v.r.a. and the d.r.a. reach the spinal cord to provide the s.a.r., perforating branches originate from these arteries and vascularize the surrounding white and gray matter.

The perforating arteries that originate from all the arteries mentioned above give off multiple branches in all directions in a tree-like pattern, and often supply two adjacent segments of the spinal cord.

4 Conclusion

Diaphanous sections of the spinal cord may offer significant advantages over the traditional and widespread use of the angiography in similar works. It allows a better correlation between angiological patterns and histology. With

this technique, we confirmed the findings previously reported (ANDERSON and KUBICEK, 1971; CAULKINS, PURINTON and OLIVER Jr., 1989; GOUAZE, CASTAING and SOUTOUL, 1965; GOUAZ'E, SOUTOUL and CASTAING, 1964; SINDOU, 1979; SOUTOUL, GOUAZ'E and CASTAING, 1965; MITCHELL, NETSKY et al., 1962; WILSON and LANDRY, 1964) about the general aspect of the arterial supply to the spinal cord of *Canis familiaris*. Despite the relatively small number of specimens studied, we were able to identify some important variations in several of these arteries that did not receive due attention in the bibliographic review we conducted. We believe some of these variations may have significant implications in the planning and interpretation of experimental procedures that involve the dog's spinal cord.

The comparatively large proportion of v.s.a. that originate from the point where the two v.a. converge (20%), or from only one of the v.a. (60%) appears to be significantly different from the usual origin of the homologous structure in the human species (HEIMER, 1995; NOLTE, 2002). This should be taken into account when inducing ischemia of the upper portion of the spinal cord in dogs in experimental settings.

As previously stated (CAULKINS, PURINTON and OLIVER Jr., 1989), we found that the anastomotic plexus on the surface of the dog's spinal cord is densest in the cervical and lumbar regions. This disposition is widely accepted as the norm in humans, and may justify that, in situations of severe hypotension, the human thoracic region is more susceptible to ischemia (SNELL, 2001).

Another relevant aspect of our study was to verify that, in the *Canis familiaris*, the segmental arteries that supply the spinal cord show remarkable variability, being globally more prevalent on the right side. In addition, caudally to the lower cervical portion of the spinal cord, they are the main responsible arteries for the supply of blood to the v.s.a. and, thus, to the ventral two thirds of the dog's spinal cord. This makes the dog a particularly useful species to simulate spinal cord ischemia in humans, as in the latter species spinal cord infarction results more often from occlusion of small segmental vessels rather than from direct commitment of the v.s.a (WINN, 2004).

In our study we found that the a.a., which is virtually always present in man, supplying most of the inferior two thirds of the spinal cord (MILEN, BLOOM, CULLIGAN et al., 1999), was present in only half of our specimens. In addition, while this artery arises in man in the vast majority of cases between T8-L2 (KOSHINO, MURAKAMI, MORISHITA et al., 1999; RODRIGUEZ-BAEZA, MUSET-LARA, RODRIGUEZ-PAZOS et al., 1991), in all the dogs we studied, this artery originated much further caudally at the level of the left fifth lumbar artery. In fact, in a large angiographic study recently performed, involving 4000 people, the most caudal origin of the a.a. was at the level of L4, and this disposition was found in only 30% of the subjects studied (LO, VALLEÉ, SPELLE et al., 2002).

Therefore, the apparently frequent absence of the a.a. and its more caudal origin, when present, may preclude the use of *Canis familiaris* as a model for ischemic injury to the caudal half of the spinal cord.

The disposition of spinal cord arteries in this species seems, however, to resemble those of humans much more closely than other species commonly used as experimental models, namely the albino rat. This last species has been reported not to have a single a.a. but 3 to 5 important thoracolumbar arteries with a function analogous to the a.a. (SCHIEVINK, LUYENDIJK and LOS, 1988). On top of this, surface anastomoses between the v.s.a. and the d.s.a. do not occur in the rat except occasionally at the caudal end of the spinal cord (TVETEN, 1976), which, again, represents a major deviation from the pattern normally found in dogs and humans.

Acknowledgements: The authors are deeply indebted to Filomena Oliveira, Augusto Batista and Erica Brázio, veterinarians at Lisbon's city kennel, for their help in providing the specimens studied, and to Carlos Lopes, laboratory technician, for his invaluable assistance in all steps of the study.

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Received June 13, 2008
Accepted January 6, 2009