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Quantitative analysis of terminal blood network in human spinal cord and progressive radiation myelopathy

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A quantitative analysis of terminal blood network in human spinal cord was done. The relative area of capillars in the gray matter is higher than in the white one and it increases from posterior to anterior spinal cord horns. The capillar area of the gray matter is larger in thoracic segments than in cervical ones. In the white matter, the largest capillar area was found in the anterior columns, the lowest in the deep parts of posterior columns. Cervical white matter has the lowest relative capillar area whereas lumbar white matter is of the largest one.
The results are compared to findings in the irradiated spinal cord. The more radiosensitive parts have been shown of having the lowest values of the relative capillar area. Possible explanation is discussed.

Key words: *Spinal cord-blood supply; capillaries; myelitis; radiotherapy – adverse effects; quantitative analysis*

Introduction

Progressive radiation myelopathy (PRM) – late reaction of the spinal cord – is a very tragic sequel of radiation treatment. Unfortunatelly, it usually arises in patients with malignancy controlled long after radiotherapy was completed. PRM was described at first by Ahlbom[1] in 1941. The diagnosis of PRM can be stated when the following criteria proposed by Pallis et al.[2] are fulfiled:[1] The spinal cord must be included in the field irradiated,[2] the main neurological lesion must be within the segments of the cord exposed to radiation,[3] the cord compression from metastasis as the cause of the neurological disorder must be excluded. PRM is one of four clinical forms of cord radiation injury according to Reagan et al:[3]

- transient myelopathy
- signs of lower neurones
- acutely developing quadru-/paraplegia
- progressive radiation myelopathy.

The PRM mechanism has not been known up to now. Didactically, three theories could be described: vascular, glial, and immunological, according to the most injured cells in volume irradiated. This injury had been suggested as a crucial in PRM pathogenesis. Because of

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the mutual functional dependence of particular cell compartments and because the radiation hits all of them randomly, the resulted radiation reaction is to be surely considered as a very complex event. Up to now, the correct relationship between radiation dose, length of cord irradiated and possible cord radiation injury (which could be very clear as being radiotherapist's daily bread) is not known.

The cervical spinal cord has been found to be more radiosensitive than the thoracic one and the lumbar cord most radioresistant. Therefore, cases of human lumbar radiation myelopathy are rather rare in comparison to the cervical and thoracic ones.[4–7]

The systematic experimental study of human PRM is not possible, therefore, we can judge a course of events by results of animal experiments. The best and the most comprehensive study of radiation effects on an animal spinal cord is Kogel's careful study[8]. His experiment conclusion is, that early lesions are caused by death of oligodendroglial cells (responsible for myelinisation) and later lesions are caused by the injury of vessels. Lower radiation doses cause later damage – primarily vascular as well. After higher doses, the earlier oligodendroglial damage appears.

The vascular component is damaged with both, lower and higher doses. Therefore, we suggested the results of the vascular spinal cord network study could be a suitable substratum for an explanation of different radiosensitivity of various parts of the cord. The spinal cord vascular supply in a human is described in detail both, macro- and microscopically.[9–11]

In literature there are papers dealing with quantitative evaluation of capillary network of various brain regions, mainly in the cortex.[12, 13] Papers describing human spinal cord vascular supply do report in general much more dense capillary network in the gray matter than in the white one.[10]

The aim of the study is to find differences in size and the density of capillary in white and gray matters between various regions in one segment and between segments of the cervical, thoracic and lumbar spinal cord.

Materials and methods

The spinal cord was studied on the spinal cords of three adult men (age of 20, 25 and 26 years). The material was gained by necropsy. After the spinal channel was opened, the whole cord was taken out and according to spinal roots it was divided in particular segments.

Tissues were fixed by 10 % neutral phosphate formol and embeded in parafin. Slices of 15 μm were stained by lucid methods (hematoxylin-eosin, cresylum violet and method by Cluver-Barrery), the impregnation by the Bodian's method and the PAS histochemical reaction was carried out as well. The best method for capillary quantitave study seems to be the method where slices were stained by the PAS reaction because capillary basic membranes are best shown.

By the quantitative evaluation of blood capillary, paraffin slices have been screened with the help of the projection microscope Pictoval (Zeiss Jena) by the magnification of 200 times on a paper and outlines of thin praecapillaries and the thinnest sections of capillaries have been drawn. Arteriolae and larger vessels have not been taken in account. A quantitative analysis has been carried out on such outlines with the semiautomatic device MOP AM O3 fy Kontron. Surface, circumference, diameter and shape factor of terminal parts of blood network have been measured. The total areas of gray and white matters in the examined spinal cord regions have been measured as well. The total number of capillaries in regions has been recalculated for the relative numbers show the density of capillaries in the area unit of 1 mm^2. Three spinal cords segments – C7, Th5 and L3-were evaluated in each spinal cord in this way.

An arrangement of blood vessels network corresponds to literature data. The difference of the density of capillary network in gray and white matters is remarkable. However, without the quantitative evaluation, there is no chance to judge any other difference. For the purpose of an evaluation of various regions in white and gray matters, the white matter area has been divided into eight regions:

- region 1. – anterior columns
- region 2. – ventral parts of lateral columns
- region 3. and 4. – lateral parts of lateral columns
- region 5. – dorsolateral parts of lateral columns encompassing pyramidal tracts
- region 6. – deep perigriseal parts of lateral columns
- region 7. – fasciculus cuneatus of posterior columns
- region 8. – fasciculus gracilis of posterior columns.

Gray matter has been evaluated according to the classification Rexed's laminas, however, nine zonas would be too detailed for our purpose. Therefore, gray matter has been divided into four levels marked with letters A, B, C, D. The central gray region, responding to the Rexed's zona X, was evaluated separately and it is marked with letter E.

Blood capillaries are put in histological preparates either on transverse or longitudinal (or moreless obligue) sections. From the point of view of a quantitative analysis, there are two inhomogeneous groups, which were evaluated separately. But for the purpose of density measurement, they were evaluated together.

Results

Dimensions of blood capillaries in the gray matter

By the evaluation of dimensions and shapes of blood capillaries, the following parameters were measured: the area of capillary section, the circumstance of capillary section, the maximal diameter and the shape factor. The information value of area and circumstance parameters were too low, therefore, results of maximal diameters and shape factors are only given here. To emphasize a difference between ventral and dorsal regions of the gray matter, the results of measurement for levels A and B (they respond to Rexed's laminas I–VI) and levels C and D (Rexed's laminas VII–IX) are given together. The range of average values describes parameters which are better observed than the average values themselves; therefore, those ranges are shown in tables. The transversal sections of capillaries are somewhat larger in ventral parts of the gray matter than in white one in all examined segments (Table 1).

Table 1. Variation range of average values of diameters of blood capillaries, which were transverally cut in examined regions of the gray matter in segments given.

Segment	Area	Variation range of blood capillaries diameters (um)
C7	A + B	13.8–15.6
	C + D	14.5–19.2
Th5	A + B	15.[illegible]–17.7
	C + D	15.5–18.1
L3	A + B	14.3–19.8
	C + D	16.3–18.9

Table 2. Variation range of average values of lengths of blood capillaries, which were longitudinally cut in examined regions of the gray matter in segments given.

Segment	Area	Variation range of blood capillaries diameters (um)
C7	A + B	27.8–53.4
	C + D	34.1–44.2
Th5	A + B	36.7–52.9
	C + D	32.1–41.7
L3	A + B	35.8–56.5
	C + D	46.5–54.8

The difference of gray matter capillary dimensions between segments are minimal, however, minimal values are in cervical segment C7, and a bit higher in thoracic segment Th5 and also in the lumbar one L3. Shape factor values are not too variable and transversely cut capillaries are in a range of 0.79–0.89. It means that the shape of capillaries is moderately oval, not too different from the regularly spherical one.

Capillary on the longitudinal section were evaluated separately, because the maximal dimension does not correspond to the maximal diameter, but to the length of it, shown in the section (Table 2). The differences between them are also not remarkable; even the length is somewhat longer in all dorsal segments of the gray matter. There are no remarkable differences between various segments, however, in both dorsal and ventral parts of the gray matter of the lumbar segment the length is longer than in the other parts.

Dimensions of blood capillary in the white matter

Substantial differences between diameters of capillaries in the transversal section and between capillary lengths in the longitudinal section in various parts of the white matter funiculi were not found. The diameters of capillaries were ranged from 13.5 μm to 24.0 μm and were mostly less than comparable values in the gray matter. The values of the shape factor were rather homogenous: 0.80–0.88.

Capillary lengths on longitudinal sections were, on the contrary, higher than in the same situation in the gray matter. Values measured in the various areas were ranged from 34.6 μm to 102.4 μm. These values were nearly two times higher than those of the gray matter. The cause of it is the radial entrance of vessels to the white matter from the vasocorona.

Blood capillaries density per area unit

The capillary network in the gray matter is much richer than in the white matter. The average values of capillary numbers per 1 mm^2 are given in Table 3 for the white matter and in Table 4 for the gray matter. Graphically, the values are shown in Figures 1, 2 and 3.

In the cervical segment C7, a higher number of white matter capillaries is found in the anterior funiculus and in the ventral part of the lateral funiculus. The highest values in the gray matter are found in the region of the ventral columna, then in the lateral one and in the dorsal part of the columna posterior. Extremely high numbers of blood capillaries were found in the central gray of all segments. This pattern is logical because the central gray is a region of entrance of aa. sulcocommissurales and their abundant branching into smaller arteriae supplying both, ventral and dorsal areas of the gray matter.

In the thoracic segment Th5, the white matter capillary density is similar to the one in the cervical segment. The highest number is found

Table 3. Average values of blood capillaries number per unit of area 1 mm^2 in various regions of the white matter of three examined segments of the spinal cord. (Description indications 1–7 see in the text).

Region of the white matter examined		C7	Th5	L3
Indication	Funiculus			
1	Funiculus anterior	33.66	41.65	36.83
2		32.18	29.64	32.33
3		28.10	24.59	32.86
4	Funiculus lateralis	23.95	26.88	29.87
5		25.13	22.31	44.01
6		28.34	28.32	44.54
	Funiculus posterior			
7	Fasciculus gracilis	25.91	26.93	32.58
8	Fasciculus ceneatus	24.57	26.90	28.34

Table 4. Average values of blood capillaries number per unit of area 1 mm^2 in various regions of the gray matter of three examined segments of the spinal cord.

Region of the gray matter examined	C7	Th5	L3
A	67.01	66.41	64.89
B	54.59	88.69	68.06
C	61.50	104.08	74.82
D	68.93	98.00	75.91
E	133.63	112.31	125.09

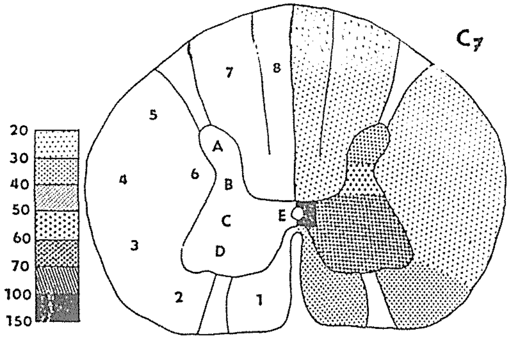

Figure 1. Density of blood capillaries per area of 1 mm^2 in various regions of gray and white matters in the seventh cervical (C7) segment.

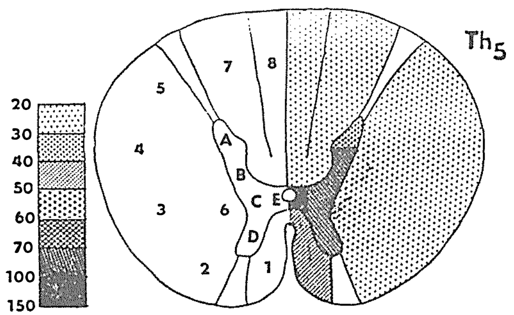

Figure 2. Density of blood capillaries per area of 1 mm^2 in various regions of gray and white matters in the fifth thoracic (Th5) segment.

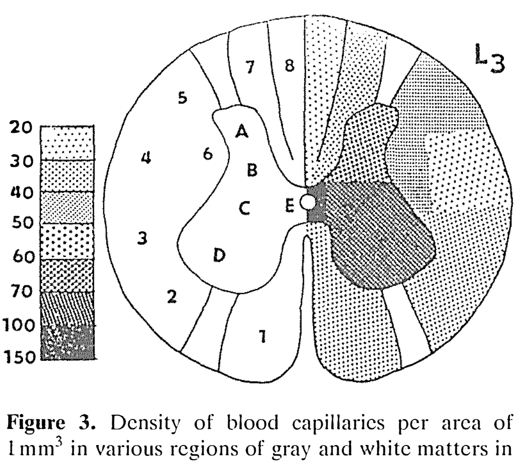

Figure 3. Density of blood capillaries per area of 1 mm^3 in various regions of gray and white matters in the third lumbar (L3) segment.

in the anterior funiculus. This number is higher than that in the cervical and lumbar segments. In the gray matter, the capillary density is significantly higher than in the other two segments except of the dorsal part of columna grisea posterior, where the density is similar.

In the lumbar segment L3, the density of capillaries is higher in both, gray and white matters. The dorsolateral and central parts of lateral funiculus have the highest density of all white matter areas studied. Density values in fasciculus gracilis and in the lateral part of the lateral funiculus were similar to the ones in the other two segments. In the gray matter of the lumbar segment a very high capillary density was found and accordingly it is divided into two parts (with Rexed' s lamina V as the border), the ventral one having a higher density value.

In all segments studied, the richest blood supply has been found in the ventral areas of the gray matter and the highest density is in the thoracic segment, next in the lumbar and relatively small number of capillaries in the cervical segment. In the white matter, no significant difference has been found between cervical and thoracic segments. On the contrary, the lumbar segment has higher values than the previous ones.

Relative area of the blood network

By the next criterion, evaluating the grade of vascularisation of various areas, the parameter of the relative area of the blood network was used. The parameter is the value expressing a proportion of all capillary areas in the whole studied area.

Results obtained for the gray matter (Table 5) confirm the previous observation. In all studied regions, the relative area of blood network increased from dorsal (A) to ventral (D) sites. The minimum value is in the cervical segment and the maximum one in the lumbar segment.

The same measurement proved the maximal proportion of the blood network in the anterior funiculus and the minimal one in the posterior funiculus (Table 6). By comparison of segments, the highest grade of vascularisation was found in the white matter of the lumbar segment and the minimal in the cervical one.

Discussion

The given results have revealed a quantitative morphological evaluation of spinal cord capillaries and the blood vessel network is a suitable method for studying the differences in blood supply of various regions. Measurements were carried out post mortem, therefore, absolute values do not respond to those in vivo values. We suggest postmortal changes as being proportional and thus the mutual comparison, which was the main goal of the paper, is possible and gives a real picture.

The gained results have confirmed a marked difference between the rich vascularisation of the gray matter and relatively not so rich in the white one. In the gray matter significant differences have been found between ventral and dorsal columns and the grade of vascularisation is increasing to the ventral parts of anterior columns. In the white matter, the vascularisation grade is usually somewhat higher in ventral funiculi. By the quantitative measurement significant diferences have been shown in various spinal cord segments as well. In the gray matter the vascularisation grade is increasing from the cervical to the lumbar and to the thoracic segment. The relative area of the vascularisation in the white matter is increasing from the cervical to the lumbar segment. The lumbar segment has significantly higher blood supply of the white matter than cervical and thoracic segments.

The papers dealing with the spinal cord radiosensitivity have claimed that the cervical cord is more sensitive than the thoracic one.[5–7, 14] More prominent histopatological changes are usually found in the white matter than in the gray one.[14–16] By lower radiation the dose changes are usually described in dorsal funiculi of the white matter, presumably in deeper parts. Further predillect regions are posterolateral superficial regions.[16] In the early stadium of late changes, the decreasing of oligodendroglial cells has been noticed. During the late phase, vascular changes (progressive changes like wall thickening, complete fibrose capillaries obliteration) become more prominent.[15] These changes could result in the necrosis of the white matter and the gray one as well. It seems that neurones are preserved intact very long.[16] This is surely the stadium when blood supply is sufficient enough to fulfil at least a minimum function. By higher radiation doses and by the late reaction, the coagulative necrosis is a predominant pattern.

Table 5. Average values of relative area of blood capillaries in per cent of the total area examined (= 100 %) in various regions of the gray matter of three given segments of the spinal cord.

Region of the gray matter examined	C7	Th5	L3
A	0.94	1.74	1.54
B	1.20	2.15	2.10
C	1.30	2.18	2.51
D	1.51	2.40	2.63
E	2.53	1.97	2.17

Table 6. Average values of relative area of blood capillaries in per cent of the total area examined (= 100 %) in various funiculi of the white matter of three given segments of the spinal cord.

Examined fasciculus of the white matter	C7	Th5	L3
Fasciculus anterior	0.79	0.88	1.21
Fasciculus lateralis	0.69	0.79	0.99
Fasciculus posterior	0.57	0.65	0.94

The different radiosensitivity of various spinal cord segments (cervical and thoracic) and different histopatological findings following the irradiation (more prominent consequences in the white matter and their predilect location in posterior and lateral funiculi) seem to be dependent on different blood supply patterns of these regions. The less is the grade of vascularisation the more radiosensitive the locations. It is contradictory because we know that oxygen sensitizes the effect of the radiation (in nondirect ionisation effect).[17, 18] In this case, one has to keep in mind we are dealing with a normal health tissue that was irradiated, but not the primarily hypoxic tumor. The hyperbaric oxygen irradiation experiments did not prove the spinal cord to be more radiosensitive.[19–21] Normally, tissues are properly supplied with oxygen; that means tissues with minimum vascularisation do not suffer from hypoxia. Where vascular network is richer, the higher need of oxygen is expected, for example by a functional demand. Such a rich capillar network could also have better capacity for the reparative process in tissues irradiated because it sufficiently provides them with oxygen and other substancies to keep them on going process. We know from our clinical practice that some late reactions are positively affected in the hyperbaric oxygen. On the other hand, some pathological situations are known with the vascular network impairment. The risk of the late radiation reaction in the central nervous system is higher by this way[22, 23].

We have to suggest the multidimensional system; the current status of it is a result of many mutually dependent processes. The nervous system is schematically composed of neurones, glial cells and vessels. Each component is a system of cells by itself including many kinds of highly specialized cells. At the same time each system is dependent on the proper function of the other complicated system. Morphologically and functionally, different heterogenous neurones without repopulation capacity are in general dependent on the very heterogenous group of glial cells that can repopulate[24, 25] and both previous systems are dependent on the proper function of the vessel system, which is again completely different from the point of view of the reparation and the repopulation.[26–28] On the other hand, not perfectly understood biological relationship of physical radiation dose, fractions number, total time of irradiation, dose rate and volume effect makes a problem more complicated.

The relation of the vascularization grade to the resultant radiosensitivity is probably only one of many relations in such a complicated and complex system as the human spinal cord, reacting to radiation, is.

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