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Relationship between the Extent of Morphological Changes in the Brain and Blood and Urine Ethanol Concentrations

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Regression analysis of the connections between changes in histological brain structures (neurons, neuroglia, and microvessels) and the extent of poisoning with ethanol (blood and urine ethanol concentrations) was performed. Specimens were obtained from 40 male and female corpses aged 20–81 years. Decreases in microvessel diameter in the cerebral cortex and cerebellum and increases in microvessel diameter in the white matter, along with increases in the numbers of vessels with stasis, in the numbers of astrocytes with cytoplasmic granularity, and in various signs of cerebral edema, as well as decreases in the numbers of astrocyte processes and the ability of erythrocytes in the stasis zone to stain with glycine cresol stain, were found to correlate with the mean square ethanol concentration (MSEC) in blood and urine. This allows this parameter to be assessed in histological studies of the brain. Conversely, knowledge of MSEC allows the presence of certain histological changes in the brain to be evaluated.

Keywords: brain, microvessels, neurons, neuroglia, ethanol poisoning.

The relevance of studies of CNS damage due to ethanol is beyond doubt [4]. Forensic medical practice currently uses a gas-liquid chromatography method for the evaluation of ethanol poisoning (EP) by assaying ethanol concentrations, usually in two biological fluids: blood and urine. However, the approved method used in practice assesses EP only in terms of the blood ethanol concentration (BEC). Assessment of the extent of EP based only on BEC, without assessment of urinary ethanol concentrations (UEC) and internal organ ethanol concentrations may not be reliable [1].

A number of investigators [5, 6] have identified parts of the brain most sensitive to alcoholemia and have noted changes to both the gray matter and the white matter, apparent as decreases in the numbers of neurons, alterations in their shapes, increases in the numbers of oligodendrocytes, and changes in neuron-glial ratios. A link was found between histological changes in the human brain with BEC and UEC, with the possibility of assessing these values [2], along with an overall measure of concentrations with a precision of up to 2‰, allowing EP to be assessed even when blood and urine levels are not measured [3]. However, this value has a 1.5 times longer scale than the usual BEC, which may disorient investigators. Furthermore, it is a product and yields a value of 0 if one factor is 0, even if the other is significant, leading to loss of information. These disadvantages are not present in an indicator consisting of the mean square of the BEC and UEC values (MSEC). It remains for morphological practice to determine the selection of any analytical solution.

The aim of the present work was to study the link between morphological changes in the brain and MSEC, to improve the evaluation of EP and find new predictors of EP.

Materials and Methods

We used specimens from 40 corpses (10 women and 30 men) aged 20–81 (mean 49 ± 1) years 12–48 h after death, obtained from the Rybinsk Interregional Department,

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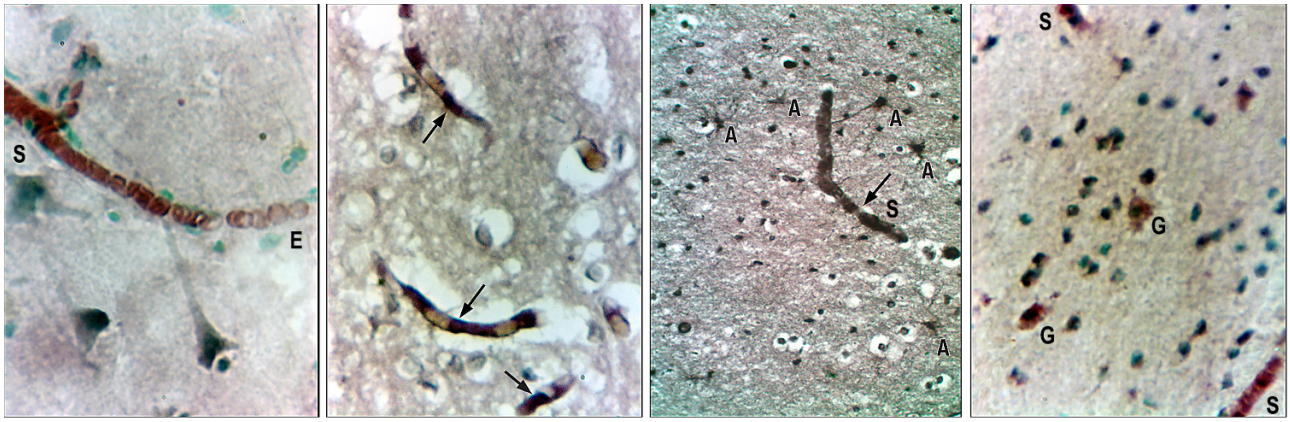

a *b* *c* *d*

Fig. 1. Brains of ethanol-abusing humans. *a*) Erythrocytes (E) and erythrocyte sludge (S) in capillaries merging at left into a venule in the cerebral cortex; *b*) erythrocyte sludge (not outlined) in venules (arrows) in the white matter, with perivascular and pericellular edema; *c*) sludge (S) in a venule (arrow), with fibrous astrocytes (A) and oligodendrocytes in the white matter; *d*) sludge (S) in a venule, with granular astrocytes (G) and oligodendrocytes mainly without edema in the white matter. Stained with glycine cresol red and methylene green. *a*, *b*, *d*) Objective ×40, ocular ×10; *c*) objective ×20, ocular ×10.

Yaroslavl Forensic Medical Expert Office. BEC varied from 0 to 6‰ (mean 1.30 ± 0.15‰) and UEC from 0 to 6.9‰ (mean 1.89 ± 0.19‰). Fragments of brain tissue were collected from different areas: the cortex of the prefrontal gyrus with subcortical white matter, the hippocampus, and the cerebellum (a total of 160 fragments). In cases with severe focal changes, specimens were taken from areas not involved in the process. Fragments were fixed in neutral formalin and cryostat sections were stained with glycine cresol red (Fig. 1).

It should be noted that almost all people dying in the state of alcoholic intoxication had long histories of alcohol consumption. This may be reflected in morphological changes in the brain and may hinder evaluation of changes arising from the most recent dose. However, it is well known that the liver is just as much a target organ for ethanol as the brain. Fatty dystrophy of the liver develops quickly in all those who abuse alcohol. We therefore also studied the liver. Fragments of liver tissue embedded in paraffin were stained with hematoxylin and eosin. Microscopic changes to the liver were evaluated in points: 1 – fatty dystrophy of the liver virtually absent; 2 – hepatocytes with fatty dystrophy occupy less than 50% of the field of vision; 3 – hepatocytes with fatty dystrophy occupy more than 50% of the field of vision; 4 – liver cirrhosis present. Data from brain tissue microscopy were analyzed using the program ImageScope Color (a digital photovideomicroscopy system) to generate a digital database containing photomicrographs from all the regions studied (up to 10 photomicrographs for each area). The program ImageTool 3.0 (on photomicrographs) was used in three microscope fields (objective ×40, ocular ×7) to count, in the white matter, astrocytes with different numbers of processes, granular astrocytes (ameboid cells with granular cytoplasm), and oligodendrocytes, both without edema and with pericellular edema, and with and without chromatolysis (lightening) of the nuclei. The presence and nature of edema of the white matter was assessed, assigning 1–3 points (1 – microvacuolar; 2 – perivascular; 3 – macrovacuolar). Studies of the gray matter involved counting the numbers of microvessels with signs of stasis (adherent erythrocytes filling the whole lumen) in microscope fields. The number of neuronophagia figures were counted in layer V of the cerebral cortex and the hippocampus and the Purkinje cell layer of the cerebellum. In addition, sludge (adherent erythrocytes) was stained in all preparations in stasis zones (codes: 100 – no stases; 101, only red stases; 102 – yellow and red stases; 103 – only yellow stases). The maximum diameters of microvessels were determined separately in the cerebral cortex and white matter. Data were analyzed in Statistica 6.0.

Results

The regression relationship was determined for the MSEC parameter: R = 0.78; corrected coefficient of determination RI = 0.58; F(9,150) = 26; $p < 0.00000$; standard error ($s_{\bar{x}}$) of regression = 1.46 (Table 1).

The regression equation for MSEC was:

$$Y = 2.15 - 0.04X_1 - 0.67X_2 - 0.65X_3 + 0.23X_4 + 0.65X_5 + 0.32X_6 + 0.41X_7 - 0.12X_8 + 0.10X_9.$$

where Y is MSEC (‰), and X_1–X_9 are the values listed in the left-hand column of Table 1, starting with age and using the coefficients given in column B.

The program took account of two factors (age and liver damage). Inclusion of these with negative values is evidence that the greater the values for these factors, the lower

TABLE 1. Regression Relationships for MSEC Variants

Parameter	β	mβ	B	mB	t(152)	P<
Free terms			2.15	0.93	2.32	0.02
Age, years	–0.22	0.05	–0.04	0.01	–4.06	8E–05
Liver damage, points	–0.32	0.06	–0.67	0.13	–5.11	1E–06
Number of A6	–0.12	0.05	–0.65	0.29	–2.27	0.02
Number of OEC	0.23	0.07	0.23	0.07	3.05	0.003
Number of GA	0.37	0.08	0.65	0.15	4.33	3E–05
Number of MSCC	0.35	0.06	0.32	0.05	5.94	2E–08
ITEW, points	0.12	0.05	0.41	0.18	2.23	0.03
MMDCC, μm	–0.12	0.06	–0.12	0.06	–2.12	0.04
MMDWM, μm	0.14	0.05	0.10	0.04	2.58	0.01

Notes. Here and Table 2: A6 – astrocytes with six or more processes; OEC – oligodendrocytes with pericellular edema and nuclear chromatolysis; GA – number of granular astrocytes; MSCC – microvessels with stasis in the cerebral cortex; ITEW – intratissue edema in the white matter; MMDCC and MMDWM – maximum microvessel diameters in the cerebral cortex and white matter respectively.

TABLE 2. Regression Relationships for MSEC Variants Excluding Age and Liver Damage

Parameter	β	mβ	B	mB	t(153)	P<
Free terms			–29.67	13.18	–2.25	0.03
Number of A6	–0.12	0.06	–0.65	0.31	–2.10	0.04
OWEC	–0.18	0.08	–0.03	0.01	–2.42	0.02
Number of OEC	0.36	0.08	0.36	0.08	4.39	2E–05
Number of GA	0.20	0.08	0.36	0.15	2.45	0.02
Staining of stasis (codes)	0.13	0.06	0.29	0.13	2.24	0.03
Number of MSCC	0.43	0.07	0.40	0.06	6.36	2E–09

Notes. OWEC – oligodendrocytes without pericellular edema and chromatolysis (lightening) of the nucleus.

the ethanol concentration required to produce a given set of histological changes in the brain. In forensic medical practice, these data are not always known to the histologist. We therefore modeled the situation by excluding these from the analysis. The regression relationships for MSEC parameters changed: R = 0.72, the corrected coefficient of determination RI = 0.51, $F(6,153) = 28$, $p < 0.00000$, standard error ($s_{\bar{x}}$) of regression = 1.6 (see Table 2).

The regression equation for MSEC was:

$$Y = -29.67 - 0.65X_1 - 0.03X_2, + 0.36X_3 + \\ + 0.36X_4, + 0.29X_5 + 0.40X_6,$$

where Y is MSEC (‰) and X_1–X_6 are the values listed in the left-hand column of Table 2, starting with A6 and using the coefficients given in column B. As the program made partial use of other indicators, the final values were not significantly degraded. The coefficient of determination decreased and the error increased very slightly, though the number of predictors decreased to six. It should be noted that neither of the equations included the number of neuronophagia figures, which were included in the regression of the combined measure of EP but in this case constituted excess information. We did not see any significant changes in neurons associated with differences in blood and urine ethanol concentrations.

Discussion

Considering the β coefficients and *t* values, MSEC was directly proportional to signs of impairments to microcirculation in the brain (the number of microvessels with stasis in the gray matter, tissue edema, and the number of oligodendrocytes with pericellular edema and nuclear chromatolysis in the white matter) and the number of granular astrocytes and inversely proportional to the number of astrocytes with more than six processes and oligodendrocytes without edema. Cytoplasm granularity has previously been regarded as mild dystrophy and is now taken as a sign of increased metabolism. Loss of processes by astrocytes may constitute damage. Changes in the microcirculation (various signs of edema) and gliocyte damage have also been noted by other authors [5, 6].

The lack of data pointing to a relationship between neuron damage and ethanol concentrations in the body is also consistent with results obtained by other investigators [7] demonstrating reversibility of changes in the nervous system after severe individual binges. We found two new facts: 1) dilation of microvessels in the white matter and constriction in the gray matter, i.e., a redistribution of blood flow, objectively protecting the cerebral cortex from the actions of the toxic agent, but promoting its hypoxia; 2) changes in the staining properties of erythrocytes (staining with glycine cresol red) in stasis zones, which, considering the properties of the complexometric indicator, is evidently linked with hypoxia and, thus, metabolic hypoxia, which is due mainly to the harmful effects of ethanol, with addition of mechanical effects (degradation of blood supply). Thus, the state of brain microvessels and gliocytes allows MSEC (the mean square ethanol concentration in biological fluids) to be determined; conversely, knowing MSEC, the presence of particular histological changes in the brain can be assessed. Detection of these changes provides for more objective assessment of the extent of EP than use of the blood ethanol concentration alone obtained during forensic chemical investigation.

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