Increase in intra-abdominal pressure raises brain venous pressure, leads to brain ischaemia and decreases brain magnesium content

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Abstract. Background. Intra-abdominal hypertension (IAH) may increase brain venous pressure, which may lead to brain injury. The aim of the present study was to analyse the effect of IAH on brain venous pressure and brain total and ionised magnesium (tMg and iMg), calcium (Ca) and zinc (Zn) contents in rats. Material and methods. Forty four adult Wistar rats were examined. Animals were divided into two groups: control, and IAH: rats with intra-abdominal pressure (IAP) elevated to 25 mmHg. IAP was measured directly in the abdominal cavity. After retrograde cannulation of the jugular vein, the jugular venous pressure (JVP) was measured as the brain venous pressure. JVP and IAP were noted after induction of anaesthesia, immediately following induction of IAH and 90 min after induction of IAH. In all rats, brains were removed for biochemical and histological analysis. Results. Biochemical analysis was performed in 30 rats, histological visualisation in 14. IAP elevated to 25 mmHg increased JVP in the IAH group. After 90 min, JVP decreased; however, its value was still higher compared with pre-IAH. In the IAH group, tMg and iMg were significantly lower than in the control group. Moreover, Ca and Zn levels were higher in the IAH group compared with the control group. The histological examination showed changes indicative of ischaemic neuronal cell stress. Conclusions. Firstly, increase in IAP elevates JVP. Secondly, raised JVP decreases tMg and iMg. Thirdly, raised JVP increases the Ca and Zn content in the rat brain. Fourthly, IAH leads to changed characteristics of brain ischaemia.

Key words: intra-abdominal pressure, intra-abdominal hypertension, brain ischaemia, brain venous hypertension, magnesium, calcium, zinc

Intra-abdominal pressure (IAP) is defined as the steady-state pressure in the peritoneal cavity [1]. The normal value of IAP ranges from 5 to 7 mmHg, but its elevation to as high as 12 mmHg is not clinically important. IAP higher than 12 mmHg is called intra-abdominal hypertension (IAH); however, organ dysfunction resulting from IAH is most frequently observed at IAP higher than 20 mmHg and is referred to as abdominal compartment syndrome (ACS).
Several studies have demonstrated numerous adverse effects of IAH [1-3]. Renal, splanchnic, cardiovascular and respiratory dysfunctions are the most notable. Additionally, many authors have reported adverse effects of IAH on the central nervous system (CNS) [5-7]. Elevated IAP displaces the diaphragm upwards, and increases pressure in the thoracic cavity. Clinical and experimental studies have shown that 20%-80% of IAP is transmitted to the thorax [8]. Increased intracranial pressure (ICP) is observed during pneumoperitoneum of 10-15 mmHg; however, significant changes are noted at IAP above 25 mmHg [6, 9]. There are at least two mechanisms that lead to brain injury following IAH. Firstly, elevation of the diaphragm following IAH increases intrathoracic pressure, leading to an increase in central venous pressure (CVP) through mechanical compression of the inferior vena cava and reduced venous return to the heart via the jugular vein [10, 11]. Decreased venous outflow from the brain impairs cerebral circulation, reduces cerebral perfusion pressure (CPP), and increases brain blood barrier (BBB) permeability, which in turn raises vascular water shift into the extracellular space resulting in cytotoxic and vasogenic oedema and extensive haemorrhagic cerebral infarction [12]. Secondly, increased IAP restricts the lumbar venous plexus, decreasing the cerebrospinal fluid (CSF) shift into veins, which results in CSF hypertension, subsequently increased ICP [13]. Irrespective of the mechanisms, IAH results in brain injury and may lead to biochemical and morphological changes in brain structures. Unfortunately, these pathologies have been not well documented. The insufflation of helium into the peritoneal cavity to induce an IAP of 20 mmHg increases some sensitive ischaemic mediators in CSF such as lactate, interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) [7]. Moreover, IAH at 20 mmHg disrupts the BBB [14]. Hence, IAH might induce the structural changes in the brain associated with ischaemia.

There are two characteristic phases of brain injury. The primary phase (early or initial) results from mechanical disruption occurring at the moment of trauma. A rapid decrease in blood and CSF outflow from the brain and an increase in intracranial pressure initiate pathologies leading to intracellular disorders. The secondary injury is defined as a biochemical disorder, initiated by the primary traumatic event [15]. Several studies have documented significant disruptions of brain ions during the secondary phase of traumatic brain injury [16-22]. A decline in brain magnesium (Mg) content (tMg and iMg-total and ionised magnesium, respectively), particularly iMg, correlates strongly with the neurological outcome [23, 24]. Additionally, the decline in brain Mg correlates with the degree of brain injury [24, 25].

A reduction in brain Mg is connected with an increase in intracellular calcium (Ca) and zinc (Zn). Significant reductions in adenosine triphosphate (ATP) synthesis result in cellular membrane depolarisation, and release of the neurotoxic amino acid glutamate, which activates the N-methyl-D-aspartate (NMDA) receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, kainic acid (KA) receptor and voltage-dependent calcium channel (VDCC). All of these enable the influx of Ca, sodium and Zn anions into the cell. Intracellular Ca influx is one of the crucial factors responsible for neuronal injury and subsequent neuronal death. Increases in cellular levels of Ca activate destructive cascades, which lead to neuronal death following the ischaemic events [19]. Likewise, Zn toxicity adversely affects the neuronal cells [20]. Increased intracellular Zn induces neuronal death via the generation of free-oxygen species, which increase superoxides and lipoperoxides [21]. In addition, high intracellular Zn activates caspases and DNA fragmentation, leading to neuronal apoptosis [22]. Importantly, these pathologies have been described after ischaemic or traumatic brain injuries. Yet the effect of disturbances in venous outflow following IAH has been not precisely elucidated. Thus, the aim of the present study was to analyse the effect of IAH on brain venous pressure, and levels of total and ionised magnesium, calcium and zinc in the rat brain.

Materials and methods

Animal preparation and instrumentation

The experimental protocol was approved by the Bioethical Committee of the Medical University of Lublin. In total, 44, adult, Wistar rats were included in our study. For the last week before the start of the study, all rats received free access to standard rat feed and water. The biochemical analysis was performed in 30 animals, which
were randomly assigned (n = 15/group) to two groups: control and IAH (rats with IAH). Prior to the examination all rats had free access to food and water without Mg supplementation. All rats were anaesthetised with a single, intraperitoneal dose of sodium thiopental (Thiopental, Sandoz, Kundl, Austria), 30 mg/kg body weight. The animals breathed spontaneously. After the induction of anaesthesia, a Veress needle was inserted into the peritoneal cavity for IAP measurements. The right jugular vein was surgically dissected and cannulated using a 24G catheter (Becton Dickinson Cannula, Helsingborg, Sweden). The jugular venous pressure (JVP) was measured as the brain venous pressure. IAH was induced by intraperitoneal air insufflations (pneumoperitoneum) to 25 mmHg, and was maintained for 90 min. The biochemical findings were supported by the histological visualisation of the frontal cortex, parietal cortex and hippocampus. For this analysis, 14 rats were randomly assigned to the control group (n = 7) and the IAH group (n = 7).

In the control group, IAP and JVP were measured after the induction of anaesthesia and the rats were then decapitated. In the IAH group, IAP and JVP were noted at three time points:
- IAH-0: after the induction of anaesthesia,
- IAH-1: just after IAP elevation to 25 mmHg,
- IAH-2: 90 min after IAP elevation to 25 mmHg.

After this time the rats were decapitated.

**Biochemical assessment**

The rat brains were removed immediately after decapitation. The brain tissue was frozen at -20°C, and 10% (m/v) tissue homogenates were prepared in 0.1 mol/L Tris-HCl buffer (pH = 7.4). Supernatants were obtained by centrifugation at 5,000×g for 15 min. The supernatant magnesium concentration was determined in the reaction with xylidine blue (Cormay-Mg 250 diagnostic kit). Absorbance was recorded at 520 nm on an Hitachi 2800 spectrophotometer (Zeiss, Jena, Germany) [26]. An ion-selective Mg²⁺ electrode (measurement range 0.2-3.0 mmol/L, accuracy ± 3%, precision within run 2% (CV) or 0.04 mmol/L (SD), selectivity logKMGNa = −3.0; logKMGK = −2.2; logKMGCa = −0.2), and a Microlite 6 analyzer (Thermo Konelab, Vantaa, Finland) were used for iMg measurements. The supernatant calcium concentration was determined in the reaction with o-cresolphthalein (Cormay Ca 120, Warsaw, Poland). Absorbance was recorded at 575 nm.

Brain Zn content was assayed by the AAS method [27]. The tissues were refrozen, dried at a temperature of 80°C for 24 h, and incinerated at 550°C for 2 h. The white ash obtained was dissolved in 25 mL of 2M HCl. Zinc concentration was measured using an atomic absorption spectrophotometer AAS-3 at 213.9 nm, with slit widths of 0.2 nm and 0.5 nm.

**Histological assessment**

Fourteen rats (seven in the control group and seven in the IAH group) were perfused transcardially with 30 mL of ice-cold saline followed by 50 mL 4% paraformaldehyde in 0.1 M, pH 7.4 phosphate buffer. Next, brains were quickly removed and fixed in buffered 4% paraformaldehyde for 24 h, at 4°C. The brains were embedded in paraffin blocks. Serial coronal sections, 5 μm thick, were cut with a microtome and stained with cresyl violet and haematoxylin & eosin. The specimens were prepared (+2.7 mm, -2.8 mm and -4.52 mm of bregma), examined and photographed under a light microscope (Optimus BX41, Japan) with a built-in Optimus Camedia C5060 digital camera (Optimus, Japan).

**Statistics**

Means and standard deviations (SD) were calculated. Student’s unpaired t-test was used for variables with normal distribution. For non-normal distributions, the Wilcoxon signed-rank and the Kruskal-Wallis ANOVA tests were applied. Additionally, Dunnett’s multiple comparison post-hoc and Spearman’s rank correlation tests were used for initial detection of inter-point differences and inter-group comparisons, respectively. Additionally, Spearman’s rank correlation test was used for overall analysis. P<0.05 was considered as significant. The power of analysis (1-β) was assessed by the G*Power test.

**Results**

The mean weight of all rats was 607.5 ± 193.27 g (553.33 ± 187.35 g and 661.33 ± 170.87 g in the control and IAH groups, respectively). There were
no differences between the control and IAH groups in weight ($P = 0.1139$). In the control group, the median values for IAP and JVP were 2 mmHg [2 and 3, quartile 1 and 3] and 3 mmHg [3 and 4], respectively. In these rats, the median value of $t$Mg and $i$Mg were 6.4 $\mu$mol/100 g tissue [6 and 6.6] and 2.8 (mol/100 g tissue [2.45 and 3.1], respectively (figure 1). In the IAH group, the median value for IAP and JVP were 3 mmHg [1.5 and 3.5] and 4 mmHg [3 and 4], respectively (time point IAH-0) (figure 1). The initial IAP and JVP in the IAH group were similar to the control group.

The intraperitoneal air insufflation to 25 mmHg increased JVP to 14 mmHg [13 and 15.5] (figure 1: IAH-1). After 90 min (IAH-2), JVP decreased to 13 mmHg [11.5 and 13.5] (figure 1). The biochemical analysis showed significantly lower $t$Mg and $i$Mg in the IAH group compared to the control group (figure 2). Moreover, both brain Ca and Zn content were significantly higher in the IAH group than in the control group.

In all rats, there were strong positive correlations between IAP and JVP ($P<0.001$; $r = 0.69$; $1-\beta = 1.00$) before the increase in IAP. The brain ionised Mg concentration correlated negatively with JVP at time point IAH-2 ($P<0.0001$; $r = -0.81$, $1-\beta = 1.00$) (figure 3). An increase in brain Zn content correlated with JVP increases ($P<0.001$, $r = 0.78$, $1-\beta = 1.00$, effect size = 0.78).

The brain structures of all rats with normal IAP (control group) are presented in figure 4.

In the IAH group, severe histological disorders were observed in three rats (44%). Mild disorders were observed in two rats (28%), and no structural changes were observed two rats (28%). In 44% of rats, an increase in IAP, and subsequently in JVP, led to significant neuronal shrinkage with perineuronal vacuolisation in the frontal cortex (figure 5A). The parietal cortex displayed the same type of lesion as the frontal cortex (figure 5B). Additionally, there were darkly stained and shrunken neurons in the granular layer of the dentate gyrus with perineuronal vacuolisation in the hippocampus (figure 5C). All pyramidal cells of the CA1 region were shrunken, with visible dendritic trees (figure 5D). In the C2 region, only some pyramidal cells were shrunken (figure 5E). The pyramidal cells of the CA3 region were morphologically normal (figure 5F). As in the C2 region, a few pyramidal cells of the CA4 region in the dentate gyrus hilus were shrunken (figure 5G).

**Figure 1.** Changes in jugular venous pressure (JVP) in the control group (C), and the group with intra-abdominal hypertension (IAH).

- Median 25%-75% Min.-Max.
- IAH-0: JVP before IAP elevation, IAH-1: In the IAH group, JVP was measured before IAH (IAH-0), immediately after IAP elevation to 25 mmHg (IAH-1), and JVP 90 min after IAP elevation to 25 mmHg (IAH-2). *** $P<0.001$: differences between IAH-0 and IAH-1, as well as IAH-0 and IAH-2, ### $P<0.01$: differences between the C and IAH-1 groups, and the C and IAH-2 groups, ○ $P<0.05$ - differences between IAH-1 and IAH-2.
Brain damage following intra-abdominal hypertension

Figure 2. Changes in brain total and ionised magnesium content (tMg and iMg, respectively), as well as brain calcium and zinc (Zn) content in rats in the control (C) and IAH groups (mean ± SD). Significant differences between the C and IAH groups in all parameters studied (P < 0.001). ††† P = 0.0007: significant differences in brain total magnesium content between the C and IAH groups, ‡‡‡ P = 0.0006: significant differences in brain ionised magnesium content between the C and IAH groups, ** P = 0.0009: significant differences in brain calcium content between the C and IAH groups, ◦◦◦ P = 0.0005: significant differences in brain zinc content between the C and IAH groups. Compared with group C, brain tMg was lower in group IAH by 15.22%, iMg by 29.35%, whereas Ca and Zn were higher by 12.82% and 31.56%, respectively.

Discussion

The present study demonstrated the adverse effects of IAH on brain structure. An increased IAP of 25 mmHg led to significant brain electrolyte disorders and histological changes indicative of neuronal cell stress. IAH reduced brain tMg and iMg concentrations and increased brain Ca and Zn concentrations. Additionally, IAH caused profound neuronal shrinking with perineuronal vacuolisation in the frontal and parietal cortex, as well as in the granular layer of the hippocampal
Figure 3. The correlation between brain ionised magnesium content and the jugular venous pressure (JVP) at time point IAH-2.

Figure 4. The control rat brain without lesions, stained with cresyl violet. A) Frontal cortex (×200). B) Parietal cortex (×200). Hippocampal formation: C) Dentate gyrus (×100). D) CA1 region (×600). E) CA2 region (×600). F) CA3 region (×600). G) CA4 region, the hilus (×600).

dentate gyrus, however such pathologies were observed only in 44% of rats.

The normal functioning of brain cells depends upon a continuous supply of oxygen and glucose for ATP synthesis because energy storage is extremely limited. Each disruption of the oxygen supply results in dysregulation of ionic homeostasis and rapid depolarisation of cell membranes leading to intracellular influxes of Na, Ca and Zn, and an efflux of Mg into the extracellular space. Magnesium plays an essential role in many intracellular processes, e.g. energy
Brain damage following intra-abdominal hypertension

Figure 5. Neuropathological changes in the brain of rat with IAH, stained with cresyl violet. A) Frontal cortex: most of the neurons are darkly stained and shrunken in appearance, with perineuronal vacuolisation (×200). B) Parietal cortex: the same type of lesion as in the frontal cortex (×100); Hippocampal formation: C) Darkly stained and shrunken neurons in the granular layer of the dentate gyrus, with perineuronal vacuolisation (×100). D) Almost all pyramidal cells of the CA1 region are shrunken with visible dendritic trees (×400). E) Some pyramidal cells of the CA2 region are shrunken (×600). F) The pyramidal cells of the CA3 region are of normal morphology (×600). G) A few pyramidal cells of the CA4 region in the hilus of the dentate gyrus are shrunken (×600).
following IAH on brain electrolyte disorders has not been demonstrated. To our knowledge, this is the first study describing brain electrolyte disorders following IAH. Based on our results we can postulate that an increase in IAP significantly impairs the venous outflow from the brain, which results in brain electrolyte disorders.

Increases in JVP resulted in severe, ischaemic, histological disorders in 44% of animals. Until recently, little information had been presented about brain pathologies following IAH and associated disturbances in venous outflow from the brain. In fact, severe brain venous hypertension caused by total superior sagittal sinus embolisation resulted in ischaemia-typical metabolic disturbances shortly after 15 min of occlusion [33]. Furthermore, IAH (20 mmHg) decreased CPP, affecting CSF and blood lactate elevation [7]. Increases in IAP to 25 mmHg caused three-fold increases in pleural pressure and significantly elevated CVP and JVP, which increased ICP to pathological values and markedly reduced CPP [34, 35]. However, some authors found a weak correlation between CVP and JVP [35], which could suggest that increased IAP reduced venous flow in jugular and vertebral veins. The occlusion of a single, dorsal, cerebral vein caused histological brain injury in 30% whereas occlusion of two veins led to histological disturbances in 90% of animals [36]. Moreover, 71% of experimental animals tolerated disturbances in venous outflow from the brain resulting from IAH, induced the histological perturbations typical of neuronal cell stress.

Although our findings are promising, two main limitations should be discussed. Firstly, we studied the effect of raised IAP on biochemical and histological changes in the brain. There is strong evidence that increased IAP affects cardiovascular function. Increases in IAP compress the inferior vena cava reducing preload with consequent reduction in cardiac output [41-43]. As a result, blood flow to the brain is impaired. For this reason, the arterial blood pressure should have been measured in our rats. A decrease in blood pressure, together with an increase in JVP, may have affected CPP. Therefore, further studies are required to confirm the effect of cardiovascular disturbances following IAH on brain injury.

Secondly, the lack of blood oxygen and carbon dioxide measurements was another limitation of our study. Raised IAP displaces the diaphragm and consequently compresses the basal lung segments, which significantly impairs the ventilation/perfusion ratio with subsequent hypoxaemia and hypercarbia [42, 43]. Moreover, an increase in IAP above 15 mmHg decreases total respiratory compliance, vital capacity and residual capacity, and increases the mean inspiratory pressure leading to severe respiratory insufficiency requiring mechanical ventilation [44]. Our animals breathed spontaneously. We could speculate that IAH at 25 mmHg suppressed the respiratory function causing hypoxaemia. The measurement of the partial pressure of arterial oxygen should have documented a degree of hypoxaemia, which might play a significant role in the development of brain electrolyte disorders.
of ischaemic brain pathology. Additionally, arteriovenous differences between partial pressures of arterial and jugular venous oxygen could show its brain consumption during IAH.

To conclude, our findings revealed that 90 min of 25 mmHg IAH resulted in biochemical and histological disturbances in spontaneously breathing rats. Such IAH increased JVP, which decreased brain tMg and iMg and increased brain Ca and Zn content. Despite the limitations described, we can confirm that an IAH of 25 mmHg causes pathology typical of neuronal cell stress.

References


