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Cisplatin-induced alterations in the blood-nerve barrier: effects of combination of vitamin B1, B6 and B12

A. Tothonglor et al., Cisplatin and vitamin B1, B6 and B12 on blood-nerve barrier

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Abstract

Cisplatin is a chemotherapeutic agent against solid cancers. However, neuropathy is a major side effect and has no effective treatment so far. Emerging evidence suggests that cisplatin might damage nerve capillaries leading to impaired blood-nerve barrier (BNB). This study aimed to investigate the ultrastructural changes of the BNB in the sciatic nerves and dorsal root ganglia of rats with cisplatin neuropathy and the effects of B1-6-12. The results showed that cisplatin 2 mg/kg injected intraperitoneally twice a week for 5 consecutive weeks caused thermal hypoalgesia and structural abnormalities of nerves and ganglia. Co-treatment with oral B1-6-12 (100:100:1) 100, 300 and 600 mg/kg/day for 5 weeks reduced the sensory deficit and structural alterations. EM analysis demonstrated the higher frequencies and wider distances of pericyte detachment in the capillaries of cisplatin than control groups. B1-6-12, especially the medium dose, reversed these abnormalities. Culture of endothelial cells and pericytes with cisplatin demonstrated reduced cell viability, increased caspase-3 activity, lower transendothelial electrical resistance and decreased expression of tight junction proteins, occludin and zonula occluden-2 (ZO-2). B1-6-12 could correct these toxic effects of cisplatin. These data confirm that cisplatin causes pathological alterations in the components of BNB which correlate with the severity of neuropathy. Furthermore, B1-6-12 is effective against these abnormalities and deserves further investigations as potential treatment for cisplatin-induced neuropathy.

Key words: pericyte, endothelial cell, cisplatin, nerve, neuropathy
INTRODUCTION

Cisplatin is an antineoplastic agent used to treat cancers of various organs [6]. One of its major side effects is peripheral neuropathy often leading to dose reduction or cessation and thus effectiveness of chemotherapy. Cisplatin-induced neuropathy is mainly characterized by sensory abnormalities in both animal models and patients [2,16,22,28]. Pathological examination showed degeneration and demyelination of nerve fibers [1,29]. Although various underlying mechanisms have been identified and relevant agents were tested, no clinically effective drugs were achieved so far [18,24].

Accumulating evidence suggests vascular dysfunction as another potential mechanism. Patients receiving cisplatin-based chemotherapy had arterial occlusion [17] and endothelial damage [5]. In cisplatin-treated rats, reduced nerve blood flow, decreased number of vasa nervorum and endothelial apoptosis were reported [15]. Recently, the lower density and detachment of nerve pericytes have been found in the rats with cisplatin neuropathy [12]. These abnormalities might impair blood-nerve barrier (BNB). This study aimed to further clarify the association of cisplatin neuropathy with BNB defects. In addition, since beneficial effects of vitamin B1, B6 and B12 in combination (B1-6-12) were seen in the preliminary study, whether this improvement in neuropathy was associated with ameliorated BNB abnormalities was also investigated.

MATERIALS AND METHODS

Animals

The experiment was approved by the institutional ethics committee (Ref. No. 19/58) and carried out in accordance with The Animals for Scientific Purposes Act 2015, Thailand. Thirty female Wistar rats weighing 250 g were divided into 5 groups: control (C), cisplatin (P), cisplatin + low-dose (P+LB), medium-dose (P+MB) or high-dose B1-6-12 (P+HB) (n=6 for each group).

Drug administration

The cisplatin and cisplatin + B1-6-12 groups received cisplatin (Pfizer, USA) diluted in normal saline to the final concentration of 0.5 mg/ml for intraperitoneal injection. The dose of cisplatin was 2 mg/kg twice a week for five continuous weeks (20 mg/kg cumulative dose). This dose regimen has been shown to induce peripheral
neuropathy in rats [2,28]. The control group received normal saline injection with the volume and schedule equivalent to the cisplatin groups. B1, B6 and B12 (all from Sigma) (100:100:1 by weight) were dissolved in normal saline and given by gavage during the cisplatin treatment once daily for five weeks. This ratio of B\textsubscript{1-6-12} was selected based on the previous studies [3,13]. Low-dose, medium-dose and high-dose B\textsubscript{1-6-12} groups received 100, 300 and 600 mg/kg/day, respectively.

**Hind-paw thermal nociception**

The details of procedure are described elsewhere [29]. Briefly, the test was done at baseline and the end of 3\textsuperscript{rd} and 5\textsuperscript{th} weeks. Each rat was placed on the hot plate analgesia meter (Harvard Apparatus, UK) maintained at 55°C. When the rat licked its hind paw on either side, elapsed time was recorded as latency. The cut-off duration of 35 s was set to avoid skin burn. The test was repeated at least 3 times with an interval of 15 min and the mean latency was obtained for each rat.

**Tissue collection**

After the last injection of cisplatin, all the rats were sacrificed by overdose anesthetics and then transcardially perfused with normal saline. This was followed by 3% glutaraldehyde. L4,5 DRG with the proximal and distal parts of sciatic nerves (divided at the trifurcation) were removed, post-fixed in 3% glutaraldehyde and embedded in epoxy resin. These specimens were used for morphometric analysis.

**Nerve morphometry**

Transverse 1 μm-thick sections of the sciatic nerve were cut, mounted on slides, and stained with paraphenylenediamine. The sections were examined under a light microscope and the cross-sectional areas were chosen using the three-window sampling method [4]. Briefly, under 40x objective lens, three windows of 0.012 mm\textsuperscript{2} were randomly placed, one in the middle and the other two in the periphery of fascicle. Images of these windows were imported into a computer via a digital camera. Morphometric analysis was done to obtain the number of myelinated fibers, axon diameter, myelinated fiber density, myelin thickness and g ratio using the Image-Pro Plus software®. The values derived from the three windows were extrapolated to the whole nerve.

**DRG morphometry**

The L4 DRG were serially cut into 2 μm-thick sections and stained with toluidine blue. The estimation for total number of neurons in each ganglion was done
using the physical dissector method. Details of the procedures were described elsewhere [28]. In brief, every 20th section was selected and the number of neurons with prominent nucleus and nucleolus was counted. Then, this number was extrapolated to the total number for the whole DRG. In addition, at least 300 neurons in each DRG were randomly analyzed for areas of the nucleus and nucleolus using the Image-Pro Plus software.

**Transmission electron microscopy**

Ultrathin sections (70 nm thickness) of the L5 DRG and sciatic nerves were stained with lead citrate and uranyl acetate. Morphology of pericytes and the basement membrane shared with endothelial cells was observed with a transmission electron microscope (JEM-1400PLUS, Japan). In each rat, 20 capillaries were randomly chosen from serial sections of each tissue (DRG, proximal and distal sciatic nerves). Each capillary was evaluated for the presence of pericyte detachment from endothelial cell and vascular basement membrane (VBM) which was classified into 2 categories: Category 1 pericyte completely attached to the VBM and endothelial cell, Category 2 pericyte detached from the VBM and endothelial cell at some points. Then, the distances at the widest detachment between the pericytes and VBM were measured in the capillaries of category 2. In addition, the thickness of VBM at the widest separation point was also measured.

**Cell culture**

Human umbilical vein endothelial cell (HUVEC) (Invitrogen) and human brain vascular pericyte (HBVP) (ScienCell) were grown according to manufacturers’ protocols. Each experiment was performed in triplicate and repeated three times. HUVEC and HBVP were divided into three groups: control, cisplatin, and cisplatin + B1-6-12. B1-6-12 was prepared in a ratio of 100:100:1 similar to the animal experiment. In cisplatin and cisplatin + B1-6-12 groups, HUVEC and HBVP were incubated with 3 µg/ml and 1.5 µg/ml of cisplatin for 24 hours, respectively. For B1-6-12 treatment, the cells were co-incubated with 1 µg/ml of B1-6-12 for 24 hours. The above doses were selected according to the preliminary data.

**MTT assay**

The MTT assay was used to evaluate the viability of HUVEC and HBVP. The cells were seeded at 1×10^4 and 5x10^3 cells/well, respectively, in 96-well plates and allowed to attach for 24 hours. The cells were then treated according to the experimental conditions for 24 hours. Finally, the cells were incubated with 100 µl MTT solution
(Life technologies, Molecular Probes, USA) for 2 hours. Subsequently, purple
formazan crystals were dissolved in 100 µl DMSO. The absorbance was measured at
570 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, USA).
The percentage of cell viability was calculated from the mean absorbance of test
samples divided by that of negative control.

**Caspase-3 assay**
Caspase-3/cpp32 colorimetric assay kit (BioVision, USA) was used to determine the
caspase-3 activity. Briefly, the cells were plated at 1x10^6 in the culture vessels. After
treatments, the cells were harvested and resuspended in cell lysis buffer. Supernatant
of each sample was collected and protein concentration was measured. Then, the
sample was diluted with cell lysis buffer to obtain the protein concentration of 1 µg/µl
and transferred to a 96-well plate. This was followed by adding working reaction
buffer and DEVD-pNA substrate. The plate was incubated at 37°C for 2 hours. The
absorbance was measured at 405 nm using a microplate reader (Multiskan GO,
Thermo Fisher Scientific, USA). The caspase-3 activity of treated cells was compared
with that of controls.

**Transendothelial electrical resistance (TEER) study**
HUVEC were cultured on the upper chamber of transwell insert (Merck, USA) which
was inserted in the 24-well plate at 1x10^4 cells/well. After the cells were grown to
confluence, they were treated according to the experimental conditions for 24 hours.
Cell resistance (R) was measured using Millicell electrical resistance apparatus
(Millicell® ERS-2, Merck, USA). TEER value was calculated using the formula:
TEER value (Ωcm^2) = (R_{sample} - R_{blank}) x membrane area (cm^2).

**Western blot analysis**
HUVEC were seeded 2 x 10^6 cells/ml in a cell culture dish. After being treated
according to experimental protocols for 24 hours, the cells were incubated on ice with
RIPA lysis buffer (Cell Signaling, USA) containing 1x protease inhibitor cocktail
(Cell Signaling, USA) for 5 min. Subsequently, the cells were scraped, centrifuged
and the supernatant collected. Protein concentration of the supernatant was
determined using PierceTM BCA protein assay (Thermo Scientific, USA). Briefly, the
sample (1.5 µg/µl) was mixed with the fluorescent dye (4:1 ratio) and denatured at
95°C for 5 min. The marker, samples, antibody diluent, primary antibody [1:200 β-
actin (Cell Signaling), 1:200 ZO-1 (Invitrogen), 1:200 ZO-2 (Invitrogen), 1:200
claudin-5 (Invitrogen), and 1:200 occludin (Invitrogen)], rabbit secondary conjugate,
streptavidin-HRP, and Luminol-peroxide were added onto the plate according to the manufacturer’s protocol. Subsequently, separation and immunodetection were conducted using WES automated western blotting system (ProteinSimple, USA). Density of digital image was analyzed using Compass software (ProteinSimple, USA). Expression of each protein was normalized to that of β-actin.

**Statistical analysis**

One-way ANOVA followed by Tukey's post hoc test was used for comparing the above parameters between the experimental groups. The test was done using SPSS for Windows version 23. Statistically significant differences were considered when p < 0.05.

**RESULTS**

**Body weight**

At baseline, the average body weight was similar between groups. However, at the 3rd and 5th weeks, the values of all groups receiving cisplatin were significantly decreased compared with that of the control group (data not shown). Food and water consumption including physical activities were similar between the cisplatin only and cisplatin + B1-6-12 groups. There was no mortality in any group during the experiment.

**Hind-paw thermal nociception**

Before the treatment, the latencies were not significantly different between groups (Fig. 1). However, at the 5th week, the latencies of the cisplatin group were significantly longer than that of the control group, indicating thermal hypoalgesia. In addition, the cisplatin + MB and cisplatin + HB groups had significantly shorter latencies than the cisplatin group and not statistically different from the control group. In contrast, the latency of the cisplatin + LB group was close to that of the cisplatin group and significantly longer than that of the control group.

**Nerve morphometry**

At the fifth week, morphometric analysis of the sciatic nerve showed that the fiber diameters of the cisplatin including cisplatin + LB and cisplatin + HB groups were significantly lower than those of the control and cisplatin + MB groups (Table 1). There were no significant differences between the cisplatin, cisplatin + LB and cisplatin + HB groups. Furthermore, the fiber densities of the cisplatin and cisplatin + HB groups were significantly higher than those of the control and cisplatin + MB groups. The values of the cisplatin + MB were not significantly different from those
of the control groups. No significant changes were observed between groups in other parameters. However, there were trends toward thinner myelin sheath in all cisplatin-treated groups and higher number of fibers in the cisplatin and cisplatin + HB groups.

**DRG morphometry**

At the fifth week, the number of DRG neurons and nuclear area were significantly decreased in the cisplatin compared with the control groups (Table 2). However, the nucleolar area was significantly decreased in all cisplatin-treated compared with the control groups. All cisplatin + B\textsubscript{1-6-12} groups had values between those of the control and cisplatin groups.

**Transmission electron microscopic analysis**

Separation between the endothelial cell and pericyte or pericyte detachment appeared to be wider and more frequent in the nerve and DRG capillaries from the cisplatin compared with the control groups (Fig. 2). The detachment was less prominent in the cisplatin + B\textsubscript{1-6-12} groups. When the number of capillaries with detachment was compared with that of total capillaries included, the ratio was significantly higher in the cisplatin than the control groups (Fig. 3). All doses of B\textsubscript{1-6-12} had the significantly lower ratio than the cisplatin group but remained higher than the control group. However, when considering data of both sciatic nerve and DRG, the ratio of the cisplatin + MB group was the least different from that of the control group. The changes in the proximal and distal parts of nerve were similar.

The separation distance of the cisplatin group was significantly longer than that of the control group in the sciatic nerves but not the DRG (Fig. 4). All cisplatin + B\textsubscript{1-6-12} groups had shorter distances compared with the cisplatin group. The values of the cisplatin + MB group were the closest to those of the control group. It is worth mentioning that only the cisplatin + HB group had significantly longer distance than the control group in the DRG. As for the thickness of basement membrane at the separation, there were no significant differences between groups (data not shown). Other pathological findings such as accumulation of lysosomes or vacuoles were not detected in the pericytes as well as endothelial cells in any group.

**Cell viability and caspase-3 activity**

Cell viability of the HUVEC was significantly reduced in the cisplatin group compared with the control group (Fig. 5). This was in agreement with the increased caspase-3 activity after cisplatin treatment. Similarly, cisplatin treatment in the HBVP resulted in lower viability and higher caspase-3 activity (Fig. 5). B\textsubscript{1-6-12} was able to
significantly enhance the viability of both HUVEC and HBVP but yielded only trends toward less caspase-3 activity in both cell types.

**Transendothelial electrical resistance**

TEER of the cisplatin group was significantly lower than that of the control group (Fig. 6). Moreover, concomitant addition of B_{1-6-12} with cisplatin caused partial restoration of the resistance.

**Expression of tight junction proteins**

Expression of occludin and ZO-2 was significantly decreased in the HUVEC exposed to cisplatin compared with the controls (Fig. 7). However, the expression of claudin-5 and ZO-1 was not significantly different. Following B_{1-6-12} treatment, the expression of occludin and ZO-2 was completely reversed to those seen in the control group. It is worth mentioning that the ZO-1 expression was significantly enhanced in the cisplatin + B_{1-6-12} group compared with the cisplatin group.

**DISCUSSION**

The rats treated with cisplatin developed neuropathy characterized by thermal hypoalgesia and morphometric changes: reduced fiber diameter, increased fiber density, loss of DRG neurons and shrinkage of nucleus and nucleolus. These features of cisplatin-induced neuropathy were similar to those previously reported [1,2,28,29]. Higher density of nerve fibers was likely due to shrinkage of fibers and slight increase in the number of fibers. Several of these functional and pathological abnormalities were comparable to those observed in cancer patients treated with cisplatin [23,27].

B_{1-6-12} had beneficial effects on thermal sensation and morphometry. High and medium, but not low doses of B_{1-6-12} could significantly reduce the prolonged thermal latency seen in the cisplatin group. Furthermore, medium dose was better than low and high doses in restoring the fiber diameter and fiber density toward controls. As for DRG, all doses of B_{1-6-12} appeared to have modest effects on the loss of neurons including shrinkage of nucleus and nucleolus caused by cisplatin. It is worth noting that B_{1-6-12} did not have any significant effect on the weight loss used to indicate the general toxicity of cisplatin.

The previous study has demonstrated the pericyte detachment from endothelial cells in the nerves from cisplatin-treated rats which was rarely seen in the controls [12]. This study confirmed with quantitative analysis that pericytes detached with significantly higher frequencies and severity in the cisplatin than in the control rats. It
is worth mentioning that despite the higher frequencies of detachment in the DRG from all cisplatin-treated compared with the control groups, the distances were not significantly different. More research is required to clarify why the pericytes detach with shorter distances in the DRG than in the nerves.

Pericyte detachment or migration has been shown in various conditions and organs. Increased migration of pericytes was observed in the retina of diabetic rats [21]. More pericytes in the anterior pituitary gland detached from the capillary walls of the prolactinoma rats than the normal controls [11]. Pericytes migrated from the vascular wall in response to traumatic brain injury [7]. Implications of the pericyte detachment are still unclear. However, since pericytes, endothelial cells and vascular basement membrane co-operate as the BNB to regulate the microvascular functions [20], the detachment is likely deleterious to the nerve. At least in the cisplatin-induced ototoxicity model, ultrastructural changes in the endothelial cells and pericyte migration in the stria vascularis of cochlea were associated with auditory impairment [30].

All doses of B_{1-6-12} could alleviate the elevation in cisplatin-induced pericyte detachment in both sciatic nerves and DRG with the best result seen in the medium-dose group. Moreover, both low and medium doses of B_{1-6-12} could equally normalize the distances of detachment. Nevertheless, high dose of B_{1-6-12} may be less favorable or even harmful, especially in the DRG. The detachment distance in the high-dose group was significantly longer than those in the other groups. This dose-dependent effect of B_{1-6-12} will be discussed later.

Due to the effects of cisplatin and B_{1-6-12} on the nerve and DRG capillaries described above, the question which cell component of the BNB was affected by the drugs has emerged. This was clarified using separate cultures of endothelial cells (HUVEC) and pericytes (HBVP). Cisplatin reduced the viability of both cell types at least via activation of caspase-3. This was in consistent with the previous reports of endothelial cell apoptosis and enhanced caspase-3 activity following cisplatin treatment [8,9,15,19]. Furthermore, TEER which represents the barrier function, was also reduced by cisplatin. Moreover, the expression of tight junction proteins in the HUVEC were examined. Expression of occludin and ZO-2, but not claudin-5 and ZO-1, was significantly lower in the cisplatin-treated cells compared with the controls. Reduced expression of tight junction proteins was also found in the stria vascularis of cochlea in cisplatin-treated mice [30].
Pericytes as well as endothelial cells are important for the normal functions of BNB and damage in any of these components might cause neuropathy. The above data from cell culture and ultrastructural analysis indicate that cisplatin causes endothelial and pericyte damage including the BNB disruption. The previous studies have already suggested the importance of tight junction proteins in the BNB integrity and nerve functions. Reduced level of claudin-5 was associated with BNB dysfunction in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) [14]. Shimizu and colleagues showed that pericytes controlled the expression of claudin-5 in the endothelial cells through secretion of growth factors [25]. Besides claudin-5, nerve pericytes also express other important components of the BNB, for example, fibronectin, collagen type IV [26].

B$_{1-6-12}$ could partially correct the reduced viability of both HUVEC and HBVP after cisplatin administration. Furthermore, the decreased TEER was also alleviated by B$_{1-6-12}$. Regarding the tight junction proteins, B$_{1-6-12}$ was able to correct the reduced expression of occludin and ZO-2 and further enhanced the expression of ZO-1 above the control level. All these data suggest that beneficial effects of B$_{1-6-12}$ on functional and morphometric parameters of cisplatin neurotoxicity described earlier might be exerted at least via improvement in the BNB functions. This could be due to less cell toxicity and enhanced expression of some tight junction proteins. Although numerous agents targeting various mechanisms were effective in experimental cisplatin neuropathy, they failed to show significant benefits in clinical trials [24]. B$_{1-6-12}$ are water-soluble vitamins essential for normal functions of the nervous system and frequently prescribed for neuropathies from various causes. However, current evidence of efficacy of B vitamins in chemotherapy-induced neuropathy is still inconclusive [18]. The results of this study support the continued effort to develop B$_{1-6-12}$ as the potential treatment for cisplatin-induced neuropathy. It is worth noting that less or unfavorable effects of high-dose B$_{1-6-12}$ in the morphometric analysis of DRG and sciatic nerves were found in this study. This might be due to toxicity of all or specific B vitamins. Excessive intake of pyridoxine (B$_6$) can cause neuropathy [10]. Therefore, optimal dose of these B vitamins must be determined to prevent the overdose side effects. Data in this study suggest the medium dose (300 mg/kg/day per oral) of B$_{1-6-12}$ as the most suitable.

The results in this study also suggest the BNB impairment as additional important mechanism underlying cisplatin-induced neuropathy. This is in accordance
with the previous study showing the BNB abnormalities in CIDP cases [14]. However, it remains to be proved whether these alterations in the BNB occur in the patients with cisplatin neuropathy. Moreover, the BNB integrity should be examined in neuropathies from other chemotherapeutic drugs or other causes. In the future, drugs with beneficial effects on endothelial cells or pericytes can be assessed for potential treatments against peripheral neuropathy with impaired BNB.

**CONCLUSIONS**

This study has demonstrated the favorable effects of B\textsubscript{1-6-12} on thermal hypoalgesia and abnormal morphometric parameters of the sciatic nerves and DRG induced by cisplatin. Ultrastructural analysis revealed that cisplatin stimulated pericyte detachment in the capillaries in those tissues. In addition, cell culture experiments showed reduced viability of endothelial cells and pericytes, transendothelial electrical resistance and expression of some tight junction proteins. B\textsubscript{1-6-12}, especially the medium dose, could improve the sensory deficit and structural alterations. Moreover, cell viability, barrier function and tight junction proteins were also corrected by B\textsubscript{1-6-12}. These data suggest that BNB disruption is one of the pathological mechanisms underlying cisplatin-induced neuropathy and B\textsubscript{1-6-12} are the potential treatment.

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| Table 1. Nerve morphometry |
|-----------------------------|
| Group | Fiber diameter (µm) | Myelin thickness (µm) | g ratio | Fiber density (µm²) | No. of fiber |
|-------|---------------------|-----------------------|----------|---------------------|--------------|
| Control (C) | 6.52 ± 0.14 | 1.29 ± 0.11 | 0.61 ± | 14,583.3 ± 557.7 | 8,798 ± 295 |
| Cisplatin (P) | 5.57 ± 0.09* | 1.21 ± 0.02 | 0.03 | 20,305.6 ± 1,073.8b | 10,026 ± |
Table 2. DRG morphometry

| Group                  | No. of neuron | Nuclear area (µm²) | Nucleolar area (µm²) |
|------------------------|---------------|--------------------|----------------------|
| Control (C)            | 21,170 ± 682  | 150.43 ± 5.64      | 14.28 ± 0.42         |
| Cisplatin (P)          | 15,581 ± 328a | 128.18 ± 4.73b     | 10.29 ± 0.62c        |
| Cisplatin + LB         | 18,113 ± 511  | 133.01 ± 4.67      | 11.40 ± 0.46d        |
| Cisplatin + MB         | 19,024 ± 766  | 137.79 ± 5.46      | 11.27 ± 0.14d        |
| Cisplatin + HB         | 19,730 ± 1,024| 130.26 ± 4.93      | 11.16 ± 0.53d        |

*p < 0.001 vs. C, p < 0.05 vs. MB, p < 0.01 vs. HB
p < 0.05 vs. C, p < 0.001 vs. C, p < 0.01 vs. C

Data are means ± SEM.

Figure 1. Changes in the thermal latency of hind paw. The graph shows means and SEM. C control, P cisplatin, P+LB cisplatin + low-dose B1-6-12, P+MB cisplatin + medium-dose B1-6-12, P+HB cisplatin + high-dose B1-6-12. * p < 0.01 P vs. C & P+HB; p < 0.05 P vs. P+MB; p < 0.05 P+LB vs. C & P+HB.

Figure 2. Representative ultrastructural images of capillaries in the sciatic nerves from the control (A), cisplatin (B), cisplatin + low-dose B1-6-12 (C), cisplatin + medium-dose B1-6-12 (D) and cisplatin + high-dose B1-6-12 (E) Arrows indicate the basement membrane shared between the endothelial cell (En) and pericyte (P). * pericyte detachment or separation between the endothelial cells and pericytes, Scale bars = 1 µm.
Figure 3. Ratio of the number of capillaries with pericyte detachment from endothelial cells to the total number of capillaries examined in the proximal and distal parts of sciatic nerve including L5 DRG. The graph shows means and SEM. C control, P cisplatin, P+LB cisplatin + low-dose B1-6-12, P+MB cisplatin + medium-dose B1-6-12, P+HB cisplatin + high-dose B1-6-12, * p < 0.001 P vs. C, # p < 0.001 P+LB vs. P & p < 0.001 P+LB vs. C, ## p < 0.001 P+MB vs. P & p < 0.01 P+MB vs. C, $$ p < 0.001 P+MB vs. P, @ p < 0.01 P+HB vs. P & p < 0.01 P+HB vs. C, @@ p < 0.01 P+HB vs. C.

Figure 4. Distance at the widest separation between the endothelial cells and pericytes in the proximal and distal parts of sciatic nerve including L5 DRG. The graph shows means and SEM. C control, P cisplatin, P+LB cisplatin + low-dose B1-6-12, P+MB cisplatin + medium-dose B1-6-12, P+HB cisplatin + high-dose B1-6-12, * p < 0.001 P vs. C, # p < 0.001 P+LB vs. P, $ p < 0.001 P+MB vs. P, a p < 0.001 P+MB vs. P, @ p < 0.01 P+HB vs. P & p < 0.01 P+HB vs. C, b p < 0.05 P+HB vs. C.

Figure 5. Cell viability and caspase-3 activity of human umbilical vein endothelial cell (HUVEC, left column) and human brain vascular pericyte (HBVP, right column) The graphs show means and SEM. Cis = cisplatin, # p < 0.01 vs. control group * p < 0.01 vs. cisplatin group.

Figure 6. Transendothelial electrical resistance (TEER) of human umbilical vein endothelial cell (HUVEC). The graph shows means and SEM. Cis = cisplatin, # p < 0.01 vs. control group * p < 0.01 vs. cisplatin group.

Figure 7. Protein expression of claudin-5, occludin, zonula occluden-1 & 2 (ZO-1 & ZO-2) Representative immunoblots are shown. The density of each protein was normalized to that of β-actin. The graphs show means and SEM. Cis = cisplatin, # p < 0.05 vs. control group * p < 0.05 vs. cisplatin group.
