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Decizie de indexare a faptei de plagiat la poziţia 00095 / 06.04.2014 şi pentru admitere la publicare în volum tipărit

care se bazează pe:

A. **Nota de constatare şi confirmare a indiciilor de plagiat** prin fişa suspiciunii inclusă în decizie.

Fişa suspiciunii de plagiat / Sheet of plagiarism's suspicion	
Opera suspicionată (OS)	**Opera autentică (OA)**
Suspicious work	**Authentic work**
OS	ARDELEAN, A.; PRIBAC, G.C.; BODO, E.; CZAPAR, M.; MOS, L. and COTORACI, C. Protective effects of mild and moderate hypothermia on cultured neuroblastoma-glioma hybrid NG108-15 cells. *Annals of RSCB*, 2009, **14**(1), p.45-50.
OA	SHIBANO, T.; MORIMOTO, Y.; KEMMOTSU, O.; SHIKAMA, H.; HISANO, K. and HUA, Y. Effects of mild and moderate hypothermia on apoptosis in neuronal PC12 cells, *British Journal of Anaesthesia. 2002.* **89**(2). p.301-305.
Incidenţa minimă a suspiciunii / Minimum incidence of suspicion	
p.45:a05 - p.45.a08	p.301:a2 – p.301:a6
p.45:a09 - p.45.a14	p.301:a10 - p.301:a12
p.45:01s – p.46:09s	p.301:05s - p.301:08s; p.301:03d – p.302:16s
p.47:Fig.1	p.303:Fig.1
p.48:Fig.2	p.303:Fig.2
p.48:Fig.3	p.304:Fig.3
Fişa întocmită pentru includerea suspiciunii în Indexul Operelor Plagiate în România de la Sheet drawn up for including the suspicion in the Index of Plagiarized Works in Romania at www.plagiate.ro	

Notă: Prin „p.72:00" se înţelege paragraful care se termină la finele pag.72. Notaţia „p.00:00" semnifică până la ultima pagină a capitolului curent, în întregime de la punctul iniţial al preluării.

Note: By „p.72:00" one understands the text ending with the end of the page 72. By „p.00:00" one understands the taking over from the initial point till the last page of the current chapter, entirely.

B. **Fişa de argumentare a calificării** de plagiat alăturată, fişă care la rândul său este parte a deciziei.

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Fişa de argumentare a calificării

Nr. crt.	Descrierea situaţiei care este încadrată drept plagiat	Se confirmă
1.	Preluarea identică a unor pasaje (piese de creaţie de tip text) dintr-o operă autentică publicată, fără precizarea întinderii şi menţionarea provenienţei şi însuşirea acestora într-o lucrare ulterioară celei autentice.	✓
2.	Preluarea a unor pasaje (piese de creaţie de tip text) dintr-o operă autentică publicată, care sunt rezumate ale unor opere anterioare operei autentice, fără precizarea întinderii şi menţionarea provenienţei şi însuşirea acestora într-o lucrare ulterioară celei autentice.	
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5.	Republicarea unei opere anterioare publicate, prin includerea unui nou autor sau de noi autori fără contribuţie explicită în lista de autori	
6.	Republicarea unei opere anterioare publicate, prin excluderea unui autor sau a unor autori din lista iniţială de autori.	
7.	Preluarea identică de pasaje (piese de creaţie) dintr-o operă autentică publicată, fără precizarea întinderii şi menţionarea provenienţei, fără nici o intervenţie personală care să justifice exemplificarea sau critica prin aportul creator al autorului care preia şi însuşirea acestora într-o lucrare ulterioară celei autentice.	✓
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10.	Preluarea identică a unor fragmente de demonstraţie sau de deducere a unor relaţii matematice care nu se justifică în regăsirea unei relaţii matematice finale necesare aplicării efective dintr-o operă autentică publicată, fără menţionarea provenienţei, fără nici o intervenţie care să justifice exemplificarea sau critica prin aportul creator al autorului care preia şi însuşirea acestora într-o lucrare ulterioară celei autentice.	
11.	Preluarea identică a textului (piese de creaţie de tip text) unei lucrări publicate anterior sau simultan, cu acelaşi titlu sau cu titlu similar, de un acelaşi autor / un acelaşi grup de autori în publicaţii sau edituri diferite.	
12.	Preluarea identică de pasaje (piese de creaţie de tip text) ale unui cuvânt înainte sau ale unei prefeţe care se referă la două opere, diferite, publicate în două momente diferite de timp.	

Notă:

a) Prin „provenienţă" se înţelege informaţia din care se pot identifica cel puţin numele autorului / autorilor, titlul operei, anul apariţiei.

b) Plagiatul este definit prin textul legii[1].

> *„ …plagiatul – expunerea într-o operă scrisă sau o comunicare orală, inclusiv în format electronic, a unor texte, idei, demonstraţii, date, ipoteze, teorii, rezultate ori metode ştiinţifice extrase din opere scrise, inclusiv în format electronic, ale altor autori, fără a menţiona acest lucru şi fără a face trimitere la operele originale…".*

Tehnic, plagiatul are la bază conceptul de **piesă de creaţie** care[2]:

> *„…este un element de comunicare prezentat în formă scrisă, ca text, imagine sau combinat, care posedă un subiect, o organizare sau o construcţie logică şi de argumentare care presupune nişte premise, un raţionament şi o concluzie. Piesa de creaţie presupune în mod necesar o formă de exprimare specifică unei persoane. Piesa de creaţie se poate asocia cu întreaga operă autentică sau cu o parte a acesteia…"*

cu care se poate face identificarea operei plagiate sau suspicionate de plagiat[3]:

> *„…O operă de creaţie se găseşte în poziţia de operă plagiată sau operă suspicionată de plagiat în raport cu o altă operă considerată autentică dacă:*
> *i) Cele două opere tratează acelaşi subiect sau subiecte înrudite.*
> *ii) Opera autentică a fost făcută publică anterior operei suspicionate.*
> *iii) Cele două opere conţin piese de creaţie identificabile comune care posedă, fiecare în parte, un subiect şi o formă de prezentare bine definită.*
> *iv) Pentru piesele de creaţie comune, adică prezente în opera autentică şi în opera suspicionată, nu există o menţionare explicită a provenienţei. Menţionarea provenienţei se face printr-o citare care permite identificarea piesei de creaţie preluate din opera autentică.*
> *v) Simpla menţionare a titlului unei opere autentice într-un capitol de bibliografie sau similar acestuia fără delimitarea întinderii preluării nu este de natură să evite punerea în discuţie a suspiciunii de plagiat.*
> *vi) Piesele de creaţie preluate din opera autentică se utilizează la construcţii realizate prin juxtapunere fără ca acestea să fie tratate de autorul operei suspicionate prin poziţia sa explicită.*
> *vii) In opera suspicionată se identifică un fir sau mai multe fire logice de argumentare şi tratare care leagă aceleaşi premise cu aceleaşi concluzii ca în opera autentică…"*

[1] Legea nr. 206/2004 privind buna conduită în cercetarea ştiinţifică, dezvoltarea tehnologică şi inovare, publicată în Monitorul Oficial al României, Partea I, nr. 505 din 4 iunie 2004
[2] ISOC, D. *Ghid de acţiune împotriva plagiatului: bună-conduită, prevenire, combatere*. Cluj-Napoca: Ecou Transilvan, 2012.
[3] ISOC, D. *Prevenitor de plagiat*. Cluj-Napoca: Ecou Transilvan, 2014.

Effects of mild and moderate hypothermia on apoptosis in neuronal PC12 cells

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Background. There is still a possibility that mild hypothermic therapy may be useful as a neuroprotective tool during the intraoperative period, although the mechanism of cerebral protection by mild hypothermia is not well understood. We hypothesized that mild hypothermia may be protective against cerebral ischaemia by inhibiting post-ischaemia apoptosis. In this study, we used serum-deprived PC12 cells as the neuronal apoptotic model and examined the direct effects of mild and moderate hypothermia.

Methods. Apoptosis was induced by depriving the cell culture medium of serum, which is one of the most representative methods to induce apoptosis, but not necrosis, in PC12 cells. Effects of mild (35 and 33°C) and moderate (31 and 29°C) hypothermia on apoptosis were evaluated. Cytotoxicity (lactate dehydrogenase leakage) and the percentage of apoptotic cells (calculated by flow cytometry with propidium iodide) were evaluated 4 days after induction of apoptosis. As a control, cells without induction of apoptosis were incubated under the same conditions as the apoptosis group.

Results. Without induction at 37°C, cytotoxicity and the percentage of apoptotic cells were over 60 and 90%, respectively. At each temperature examined below 35°C, significant decreases in cytotoxicity and the percentage of apoptotic cells were observed. Mean cytotoxicity at 31 and 29°C was 50.2 (SD 4.2)% and 47.9 (4.4)%, respectively. The percentage of apoptotic cells at 31 and 29°C was 42.5 (7.4)% and 36.5 (7.3)%, respectively. In the control group, cytotoxicity and the percentage of apoptotic cells were significantly higher at 29°C than at 37°C.

Conclusions. Mild and moderate hypothermia (29–35°C) inhibited apoptosis, although hypothermia below 30°C may induce apoptosis in intact cells.

Br J Anaesth 2002; **89**: 301–5

Keywords: cells, apoptosis; brain, cerebral ischaemia; hypothermia

Accepted for publication: April 9, 2002

Hypothermia for cerebral protection was first introduced in the 1950s.[1 2] Prior to this, hypothermic treatment was not widespread due to its harmful effects, including cardiovascular and respiratory depression and severe infection. However, Busto and colleagues reported that a small reduction of intraischaemic rat cerebral temperature (from 36°C to 34°C) markedly attenuated ischaemic cell damage over 20 min in the four-vessel occlusion model.[3] Although a recent multicentre randomized controlled trial questioned the effectiveness of mild hypothermic therapy in patients with severe brain injury,[4] there is still a possibility that mild hypothermic therapy may be useful as a neuroprotective tool during the intraoperative period.[5 6]

The mechanism of cerebral protection by mild hypothermia is still unclear. However, it is not wholly attributable to metabolic inhibition, because a temperature reduction of 1–2°C decreases the cerebral metabolic rate of oxygen consumption by only 7–14%.[7] In fact, Busto and colleagues also reported that cerebral energy metabolites such as ATP were depleted to a similar degree at 33, 34 and 37°C at the end of 20 min of four-vessel occlusion.[3]

Recently, it was reported that apoptosis was detected following focal cerebral ischaemia, especially in the penumbral region.[8 9] It has also been reported that delayed neuronal death following global ischaemia may be partly attributable to apoptosis.[10 11] Accordingly, it is speculated that ischaemic cell death is partly due to apoptosis in addition to necrosis.

Necrosis occurs due to intracellular energy depletion, after which metabolic depression may inhibit necrosis. In contrast, apoptosis occurs due to activation of intracellular cascades.[12] Therefore, mild hypothermia may protect against cerebral ischaemia by inhibiting the intracellular apoptotic cascades activated by ischaemia. In this study, therefore, we used serum-deprived PC12 cells as the neuronal apoptotic model and examined the direct effects of mild and moderate hypothermia on apoptosis.

Methods

Cell culture and induction of apoptosis

PC12 cells, which were originally derived from rat pheochromocytoma, were provided by Riken Gene Bank (Tsukuba Science City, Japan). Cells having undergone up to 10 passages from the original cell line were used in the experiments. PC12 cells were maintained on collagen-coated dishes (Biocoat Cellware, Bedford, MA, USA) in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA), supplemented with 5% heat-inactivated fetal bovine serum (MoreGate, Melbourne, Australia) and 5% heat-inactivated horse serum (Gibco), at 37°C in a 100% humidified atmosphere containing 5% carbon dioxide/95% air. Apoptosis was induced by depriving the medium of serum, which is one of the most representative methods of inducing only apoptosis in PC12 cells.[13] We have previously demonstrated that apoptosis, but not necrosis, is induced in our cells by serum deprivation through DNA electrophoresis and fluorescence microscopic analyses.[14]

Effects of hypothermia: cytotoxicity assay

Cells (1×10^5) were subcultured to 35-mm collagen-coated dishes. Two days after normal culture at 37°C, apoptosis was induced in phenol-red-free DMEM (Gibco) without serum. The cells were randomly incubated in chambers, in which the temperature was maintained at 37, 35 or 33°C (mild hypothermia series), or 37, 31 or 29°C (moderate hypothermia series), in a 100% humidified atmosphere containing 5% carbon dioxide/95% air. The temperature of each chamber was accurately maintained by a thermostat (Digimulti D611; Techno Seven, Yokohama, Japan). As a control, cells in which apoptosis was not induced were incubated under the same conditions.

Cytotoxicity was evaluated 4 days after serum deprivation using the lactate dehydrogenase (LDH) leakage assay as reported previously.[14] Briefly, the culture supernatants were collected after the cells were sedimented by centrifugation. Next, they were incubated with a LDH reaction mixture using a cytotoxicity detection kit (Boehringer Mannheim, GmbH, Mannheim, Germany). Changes in absorbance at 490 nm were measured with a spectrophotometric microplate reader (model 3350; Bio-Rad, Hercules, CA, USA). LDH activity of each sample was obtained from the change in absorbance of the LDH standard. LDH activity of the cells was also measured after cells were lysed in 1% Triton X-100. Cytotoxicity (%) was defined as follows: (LDH activity that leaked from the cells)/(LDH activity that leaked from the cells + LDH activity within the cells).

Effects of hypothermia: percentage of apoptotic cells

Cells (1×10^5) were subcultured to 35-mm collagen-coated dishes. Two days after normal culture at 37°C, the medium was deprived of serum. The cells were randomly allocated into a mild or moderate hypothermia series as in the cytotoxicity assay. As a control, non-induced cells were incubated under the same conditions.

The method for flow cytometric analysis was as described previously.[14] Briefly, cells were fixed in 70% ethanol following harvest. Fixed cells were incubated with 0.4 mg ml^{-1} RNase A (Sigma, St Louis, MO, USA) and stained with propidium iodide (Sigma). Cellular DNA content was measured by flow cytometry (FACS Calibur™; Becton Dickinson, San Jose, CA, USA). Calculation of the percentage of apoptotic cells was based on the cumulative frequency curves of the appropriate DNA histograms. Apoptotic cells were regarded as the population that contained less DNA than at the G_1 peak (Fig. 1).

Statistics

Values are expressed as mean (SD). To compare cytotoxicity and the percentage of apoptotic cells, one-factor analysis of variance (ANOVA) was used. When a significant difference was observed, post hoc analysis was performed with Sheffe's test. Statistical significance was assumed when $P<0.05$.

Results

Cytotoxicity: apoptosis group (Fig. 2)

Cytotoxicity at 37°C was >60% 4 days after induction. At each temperature below 35°C, cytotoxicity decreased significantly ($P<0.01$) compared with 37°C.

Cytotoxicity: control group

We performed 10 and nine experiments in the mild and moderate hypothermia groups, respectively. Cytotoxicity at

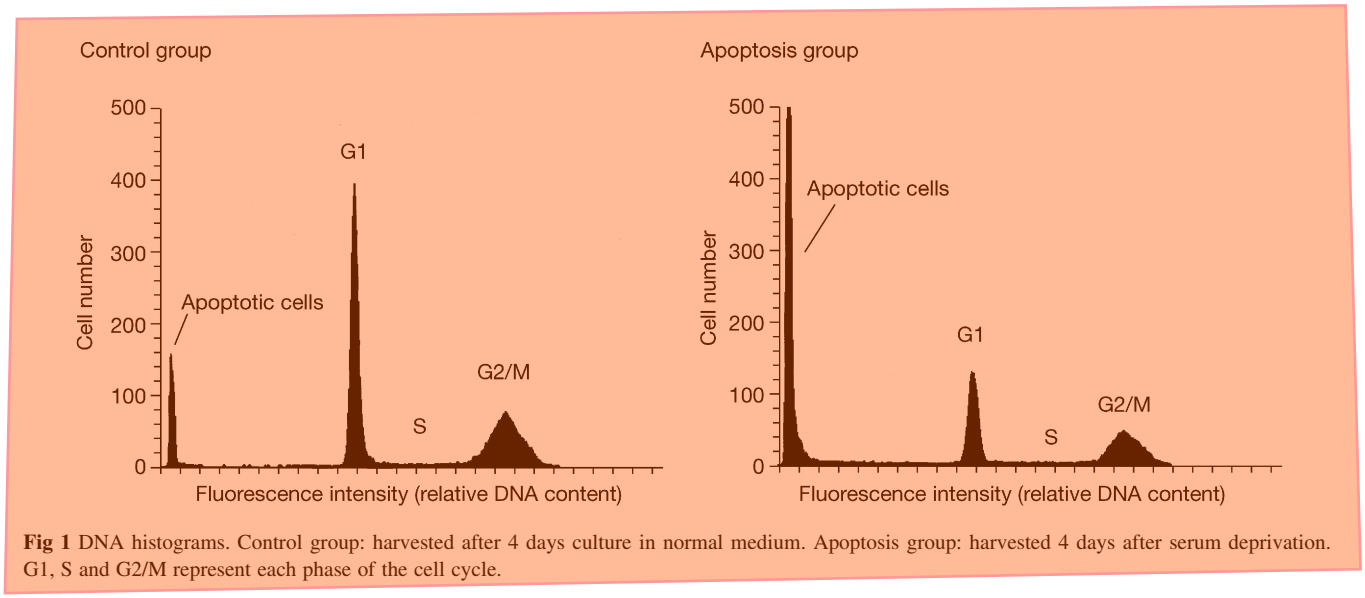

Fig 1 DNA histograms. Control group: harvested after 4 days culture in normal medium. Apoptosis group: harvested 4 days after serum deprivation. G1, S and G2/M represent each phase of the cell cycle.

37, 35, 33 and 31°C was approximately 10–11%. At 29°C, cytotoxicity was 17.0 (5.8)% and was significantly higher than that at 37°C (P=0.03).

Percentage of apoptotic cells: apoptosis group (Fig. 3)

The percentage of apoptotic cells at 37°C was >90%. At each temperature below 35°C, this was significantly decreased compared with 37°C (P<0.01) (Fig. 3). At 29°C, this value decreased to 36.5 (7.3)%.

Percentage of apoptotic cells: control group

We performed 20 and 17 experiments in the mild and moderate hypothermia groups, respectively. At 37, 35, 33 and 31°C, the percentage of apoptotic cells was <10%. This value was 12.3 (4.2)% at 29°C and was significantly higher than that at 37°C (P<0.01).

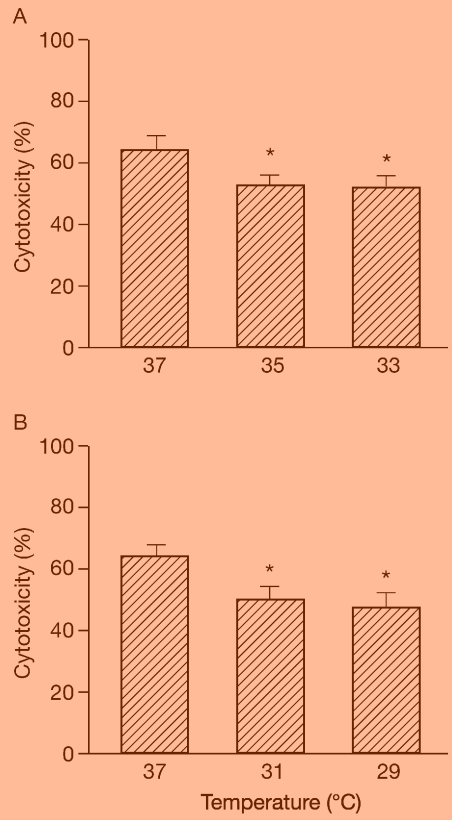

Fig 2 Effect of hypothermia on cytotoxicity in the apoptosis group. Values (SD) indicate cytotoxicity (lactate dehydrogenase leakage) in the mild (n=7) (A) and moderate (n=10) (B) hypothermia groups. A significant difference is shown among the mild and moderate hypothermia groups (P<0.01). *P<0.01 compared with 37°C.

Discussion

Several hypotheses have been suggested with regard to the neuroprotective effect of mild hypothermia. For example, depression of the glutamate surge,[15] delayed onset of intracellular calcium mobilization,[16] inhibition of reactive oxygen species (ROS) production[17] or early recovery of protein synthesis[18] have all been reported. However, the exact mechanism has not been elucidated. Recently it has been reported that cerebral apoptosis is decreased under hypothermia at 33°C after forebrain and focal ischaemia in rats.[19 20] Bossenmeyer-Pourie and colleagues reported that hypothermia at 32°C reduced apoptosis following 6 h of hypoxia in neuronal cultures from the forebrain of 14-day-old rat embryos.[21] However, necrosis in addition to apoptosis was observed in their model so that the effect of hypothermia on apoptosis alone was not clarified. In addition, the effects of several temperatures were not examined. To our knowledge, the direct effects of mild and moderate hypothermia on apoptosis, but not necrosis, are not well understood. Cell survival by hypothermia itself has not been studied extensively.

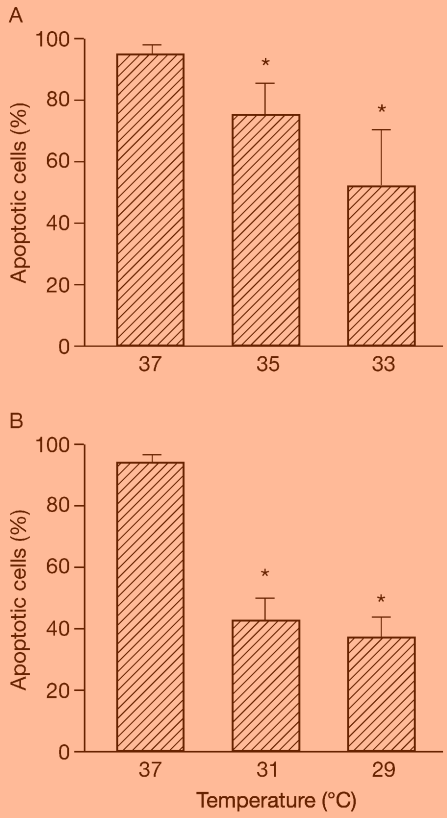

Fig 3 Effect of hypothermia on percentage of apoptotic cells in the apoptosis group. Values (SD) indicate percentage of apoptotic cells in the mild (n=18) (A) and moderate (n=13) (B) hypothermia groups. A significant difference is shown among the mild and moderate hypothermia groups (P<0.01). Hypothermia (35, 33, 31 and 29°C) significantly inhibited apoptosis compared with 37°C (*P<0.01).

Studies of cytotoxicity and percentage of apoptotic cells revealed that hypothermia directly inhibited neuronal apoptosis in the mild and moderate range (29–35°C). As we reported previously,[14] several mechanisms such as production of ROS and induction of caspase activity are suggested as intracellular cascades leading to apoptosis induced by serum deprivation in PC12 cells. They are also observed after cerebral ischaemia.[22–24] Kil and colleagues examined the levels of the salicylate hydroxylation product as an index of ROS production in the rat forebrain ischaemia model at brain temperatures of 30, 36 and 39°C.[17] Levels of the salicylate hydroxylation product significantly decreased in a temperature-dependent fashion. It was reported that expression of caspase-3 proteins was decreased during hypothermia at 33°C after rat forebrain ischaemia.[19] Thus, the inhibition of apoptosis under hypothermia observed in this study might also be related to depression of such intracellular cascades.

Lucas and colleagues reported that there were no morphological or electrophysiological changes in spinal cord neurone culture, even when the temperature was decreased to 17°C for 2 h.[25] In our study, however, cytotoxicity and the percentage of apoptotic cells significantly increased at 29°C in the control group. This result suggests that hypothermia may damage intact cells through induction of apoptosis if the period of hypothermia is lengthy, even if the range of hypothermia is moderate. The result may be noteworthy because as of late, hypothermic therapy is usually performed for several days.

In conclusion, we evaluated the relationship between hypothermia and neuronal apoptotic processes using PC12 cells, in which apoptosis but not necrosis was induced by serum deprivation. Mild and moderate hypothermia (29–35°C) inhibited apoptosis, although hypothermia below 30°C may induce apoptosis.

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