Experimental Article

Online ISSN : 2314-8969
Print ISSN: 2314-8950
www.esa.org.eg

Egy Spine J 8:42-48, 2013

The effect of phosphatidylcholine/ deoxycholate on nervous tissues: A histopathological study

Hanan El-gowelli MD,[1] Bassma Elsabba MD,[2] Hisham Elsaghir MD,[3] Emad Yosry MD[3]

Departments of Pharmacology and Toxicology,[1] Pathology,[2] and Orthopedic Surgery,[3] Faculty of Medicine, Faculty of Pharmacy, Alexandria university, Egypt.

Abstract

Background Data: Phosphatidylcholine/deoxycholate is now widely used in injection lipolysis mainly for aesthetic purposes inducing lipolysis in subcutaneous fat for body contouring and also for shrinking small subcutaneous lipomas. In this work, the authors discuss the effects of phosphatidylcholine dissolved in deoxycholate on nervous tissues in rats to assess the hazards of injection lipolysis in treatment of spinal lipomas as an alternative to surgery.

Purpose: to highlight the histopathological effects of phosphatidylcholine on neurological tissues of a rat model to determine whether it is safe to use it as a mesotherapeutic agent for treating spinal lipomas as an alternative for surgery.

Study design: a prospective histopathological study on rat model.

Material and Methods: 12 young female Wistar rats (2 month old) were divided equally into 2 groups. The treatment group was injected percutaneously by Lipostabil® (0.1 ml/rat/day containing 50 mg/ml phosphatidylcholine dissolved in deoxycholate) in the groin area infiltrating the femoral bundle and the control group was injected with 0.1 ml/rat/day normal saline. The injection was repeated for 4 successive days. Biopsies were harvested on the fourth day from the femoral bundle and studied by light microscopy. The pathology was scored semi-quantitatively for inflammation, necrosis, fibrosis, and nerve damage.

Results: Repeated injection of phosphatidyl choline deoxycholate caused intense inflammation adjacent to the nerve leading to neural damage, deposition of collagen fibers amongst the inflammatory background as an early sign of fibrogenesis and tissue necrosis

Conclusion: The current data highlights the risk of using such combinations near nerves not only spinal lipomas for its intense inflammatory and necrotic effects. (2013ESJ056)

Key words: phosphatidyl choline, spinal lipoma, nerve tissue

Introduction

Spinal lipomas are benign tumors with a peak presentation between 10 and 40 years. Nevertheless, they have aggressive manifestations related to mass effect and secondary compressive myelopathy resulting in progressive pain, autonomic and sensorimotor deficits.[1,21]The slow growth of spinal lipomas can be tolerated being accommodated within the spinal canal all for a point where there is no space left to accommodate it, and hence compressive manifestations will take place.[2,4]

Surgical treatment of spinal lipomas in symptomatic cases consists of untethering of the cord along with de-bulking of the lipomatous mass rather than total excision.[8] De-bulking was agreed amongst most neurosurgeons because total excision necessitates more aggressive and manipulative technique.[15,16]Nonetheless, recurrence rate becomes high due to hyperplasia of residual adipocytes,[10] and that is just to say the least about the surgical treatment when other more warning complications such as spinal injury, deprivation of the cord from blood supply through separation of the cord from the lipomatous mass, wound breakdown, CSF leakage and pseudomeningocele takes place.[4,12]

Deoxycholic acid (DC) is a natural emulsifier and a secondary bile acid that results from the metabolism of a primary bile acid called cholic acid by the intestinal bacteria.[3]On the other hand, phosphatidylcholines (PCs) are a class of phospholipids that incorporate choline. They are major component of biological membranes and can be easily obtained from a variety of readily available sources such as egg yolk or soy beans. PCs are insoluble in water, and thus require an emulsifier, traditionally DC, to solubilize them.[9,24] PC/DC combination was approved for IV treatment of fat embolism in Germany[12]and is now widely used as an alternative for liposuction for reduction of subcutaneous fat.[7] Because the extensive nature of intra-spinal lipomas and their complex relationship to neural elements makes the surgical option for the removal of spinal cord lipomas challenging, this work aimed to assess a less invasive path for the removal of lipomas via lipo-dissolution using PC/DC combination and to investigate the effect of this combination on neural tissues.

Materials and Methods

Animals:

Twelve female Wistar rats (Faculty of Pharmacy, Alexandria University, Alexandria, Egypt) weighing 90 to 110 g were used in this study. All experiments were performed in strict accordance with institutional animal care and use guidelines.

Materials:

Phosphatidyl choline (50 mg/ml) derived from soy bean Lecithin dissolved in deoxycholate (Lipostabil ® N by A. Nattermann & Cie. GmbH Germany, member of the Sanofi-Aventis Group); normal saline (Sodium Chloride 0.9% w/v in sterile filtered double-distilled water, without any additives) (El-Nasr Pharmaceutical Chemicals Co ADWIC®); and Thiopental® (Sandoz, Basel, Switzerland) were purchased from commercial vendors.

Experimental protocol:

Under aseptic technique, rats were injected percutaneously with 0.1 ml/rat saline (Control group, N=6) or by 0.1 ml/rat of Lipostabil ® N (PC/DC group, N=6) in the groin of the right side of the rat near the femoral nerve for 4 consecutive days. Injections were made using sterile 30-gauge needles.

On the fourth day, rats were anesthetized using thiopental (50 mg/kg) administered via intra-peritoneal injection. Gentle dissection of the femoral bundle was performed and about 1 cm of the bundle was excised and flushed with ice cold saline. Specimens were immediately immersed in neutral buffered formalin for light microscopy study. All rats were euthanized with overdose of the anesthetic. The biopsies were stained with Haematoxylin and Eosin stain (H&E) and examined by light microscope.[6]

Evaluation of all four categories (inflammation, fat necrosis, fibrosis, and nerve damage) was done in a semi-quantitative manner for H & E stained slidesunder light microscope. Each category was scored into one of four grades in one microscopic field (×100): grade 1 (<25%), grade 2 (26% to 50%), grade 3 (51% to 75%), and grade 4 (76% to 100%).[17,20]

Statistical Analysis:

Data are expressed as means ±SEM. Simple means are analyzed by unpaired t-test. The analysis was performed using Graph Pad Prism, software release 3.02. Probability levels less than 0.05 were considered significant

Results

Table 1 and 2 and figures (1-4) summarize the histopathological scoring of tissues inflammation, fat necrosis, fibrosis and nerve damage. Compared to the control group injected with saline, injection of 0.1 ml Lipostabil® for 4 consecutive in the groin of the right side of the female Wistar rats near the femoral nerve caused significant intense leukocytes infiltration in the skeletal muscles causing severe inflammation and muscle damage leading to the deposition of collagen fibers as an early sign of fibrosis. These leukocytes cuffed the nerve bundle causing significant neural damage. The intense inflammation caused significant necrosis in the adipose tissue and skeletal muscles.

Table (1). Effect of local normal sterile saline injection (0.1 ml/rat for consecutive 4 days) on different tissues at the injection site, control.

Saline	Inflammation	Necrosis	Fibrosis	Nerve damage
1 Rat	2	1	1	1
2 Rat	1	0	0	0
3 Rat	1	0	0	0
4 Rat	2	0	0	0
5 Rat	1	0	0	0
6 Rat	1	0	0	0
Min	1	1	1	1
Max	2	1	1	1
Mean±SD	0.52±1.33	0.41±0.17	0.41±0.17	0.41±0.17

Values are expressed as means ± SD of 6 observations.

Table (2).Effect of local Lipostabil® injection (0.1 ml/rat for consecutive 4 days) on different tissues at the injection site, treated

Lipostabil®	Inflammation	Necrosis	Fibrosis	Nerve damage
1 Rat	3	2	0	2
2 Rat	1	2	0	1
3 Rat	4	3	2	2
4 Rat	2	1	2	1
5 Rat	3	2	1	2
6 Rat	2	2	2	1
Min	1	1	0	1
Max	4	3	2	2
Mean± SD	*1.05±2.50	***0.63±2.00	*0.98±1.17	***0.55±1.50
P Value	0.0345	0.0001	0.0438	0.0008

Values are expressed as means ± SD of 6 observations. * Denotes P<0.05 vs. corresponding control (saline) values. *** Denotes P<0.001 vs. corresponding control (saline) values.

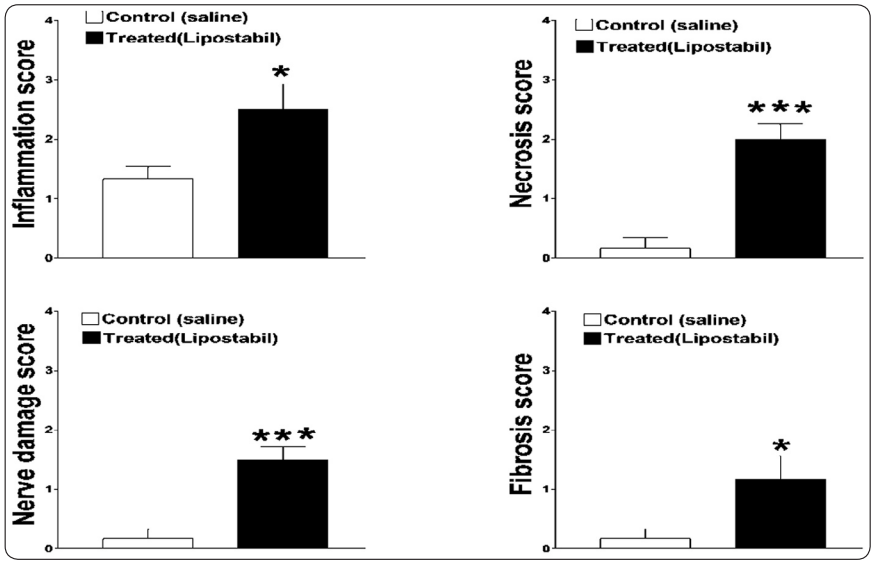

Figure 1. Effect of local Lipostabil® injection versus saline on different tissues at the site of injection**.** Bar graphs showing histopathlogical tissue changes due to local injection of Lipostabil® (0.1 ml/rat) for 4 consecutive days compared to saline (control).Values are expressed as means SD of 6 observations. * Denotes P<0.05 vs. corresponding control (saline) values. *** Denotes P<0.001 vs. corresponding control (saline) values.

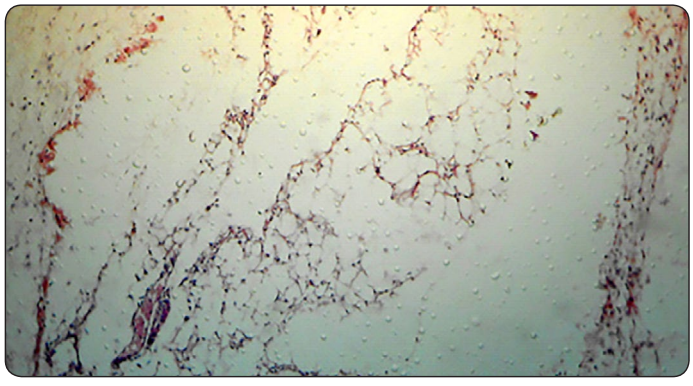

Figure 2. Effect of Repeated Lipostabil® Injection on adipose tissue at the Injection Site. Photomicrographs (100 ×) of adipose tissue from rats injected with Lipostabil® (0.1 ml/day/rat) for 4 consecutive days. The section is stained with H and E stain. The photomicrograph shows ruptured fat globules and fat cysts, an indication of fat necrosis.

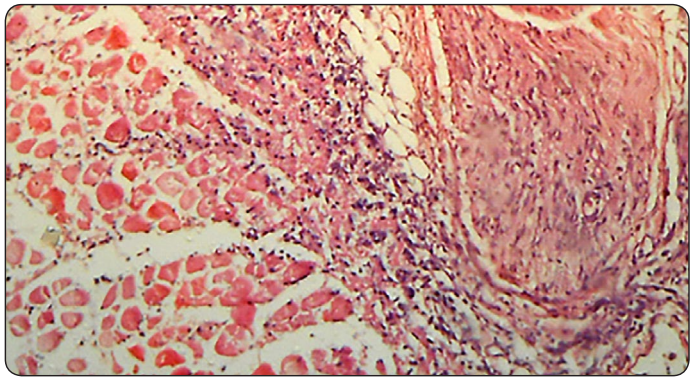

Figure 3. Effect of Repeated Lipostabil® Injection on Neural Tissues at the Injection Site. Photomicrographs (300 ×) of nerve bundle stained with H & E stain from female Wistar rats injected with 0.1 ml/day/rat Lipostabil® for 4 consecutive days showing nerve bundle entrapped within intense inflammation at the site of injection.

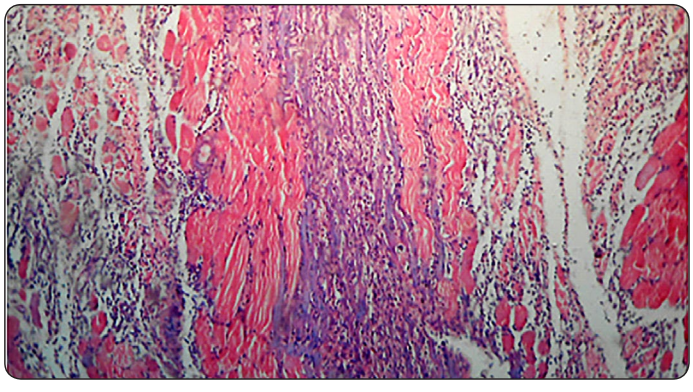

Figure 4. Effect of Repeated Lipostabil® Injection on skeletal muscles at the Injection Site. Photomicrographs (200 ×) of skeletal muscles stained with H & E stain from female Wistar rats injected with 0.1 ml/day/rat Lipostabil® for 4 consecutive days showing prominent myofiber necrosis, myophagocytosis, and regeneration at the site of injection.

Discussion

Compared to the control group injected with saline, injection of 0.1 ml Lipostabil® for 4 consecutive in the groin of the right side of the female Wistar rats near the femoral nerve caused significant lipolysis in the inguinal fat compared to the control group receiving only saline injections. This was associated by the formation of fat cysts as well as fat necrosis. There was also intense leukocytes infiltration in the skeletal muscles causing severe inflammation and muscle damage leading to the deposition of collagen fibers as an early sign of fibrosis. Such results are consistent with previous studies where local injection of different doses of PC/DC in the abdominal cavity of rats or human volunteers resulted in dose-dependent reduction in cell membrane integrity of adipocytes and increased fat cyst formation.[23]

It is presumed that the formation of fat cysts and fat necrosis following PC/DC injection can be the result of local irritation and intense inflammatory reaction induced by PC/DC injection combined with the lipolytic effect of the formula. In support of this notion, in the current study the group treated with Lipostabil® injection showed significant intense infiltration of leukocytes at the injection site compared to the control group treated with saline, which is considered as a sign of local inflammation due to the injection of PC/DC. This inflammation can contribute to adipocytes death and fat necrosis. Clinically, in 2013, Reeds et al,[19]reported that in injection of PC/DC in one side below the umbilicus in women with body mass index above 30 caused significantly reduction in the thickness of the anterior subcutaneous abdominal fat. However, the adipose tissue showed rapid increases in crown-like structures and macrophage infiltration suggesting that PC/DC injections can effectively reduce abdominal fat volume and thickness by inducing adipocyte necrosis.

It is worth noting that PC/DC formulation can induce the lysis of various cell types including adipocytes, normal human fibroblasts, endothelial cells, and skeletal muscle cells in a nonspecific manner.[11] Indeed, the role of DC in the development of fibrosis at the injection site cannot be overruled. Previous studies showed that increased serum level of bile acids in cholestasis can cause hepatic fibrogenesis.[25] Therefore, it is presumed that the extensive infiltration of inflammatory cells in the muscle fibers at the site of inject along with the DC present in the Lipostabil® injection can be responsible, at least partially, for the activation of fibrotic cascade at the injection site.

The effect of PC/DC in neural tissues was not studied in depth before. Studies used DC experimentally to breakdown the blood brain. The breakdown of the blood brain barrier can subject the brain tissues to noxious substances causing neural damage.[5] Nonetheless, the direct effect of DC on the neural tissues, to our knowledge, was not studied. The reported side effects of injection of PC/DC formulations include increased sensitivity to pain in areas of lipo-dissolution treatments,[13,26] which could be the result of neural injury at the site of injection. Also, inflammation is believed to sensitize the nerve to all incoming stimuli.[14,22] In such a state, even minor mechanical stimulation of the nerve can evoke severe exaggerated pain.

Conclusion

PC/DC combination was shown in this work to have damaging effect on nerves as well as other structures such as skeletal muscles and conveying lipolysis in an intense inflammatory and necrotic pathway. Therefore, its usage in the current form carries a high risk/benefit ratio that can't be overlooked. Indeed, this study casts even a darker shadow on PC/DC generally as FDA yet to approve it. The current study is a basic lab research that directs its future counterparts to go and find alternatives for surgery role in spinal lipomas and other sites of lipomas as well. As for PC/DC in its current form and drug delivery system, it is uncertain that it can have a role in the management of spinal lipomas. Also, it should be encouraged to find a way to split PC from DC for getting rid of the possible detergent effect of DC.

References

1. Bancroft LW, Kransdorf MJ, Peterson JJ, O'Connor MI: Benign fatty tumors. Skeletal Radiol 35:719-33, 2006
2. Bhatoe HS, Singh P, Chaturvedi A, Sahai K, Dutta V, Sahoo PK: Nondysraphic intramedullary spinal cord lipomas: a review. Neurosurg Focus 18:ECP1, 2005

3. Björkhem I, Einarsson K, Melone P, Hylemon P: Mechanism of intestinal formation of deoxycholic acid from cholic acid in humans: evidence for a 3-oxo-delta 4-steroid intermediate. J Lipid Res 30:1033-9, 1989
4. Blount JP, Elton S: Spinal lipomas. Neurosurg Focus 10:e3, 2001
5. David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A: Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis?. J Neurosci 29:10588-99, 2009
6. Drury RAB, Wallington EA: Carleton's Histological Techniques. 5thed. New York: Oxford University Press, 1980
7. Duncan DI, Hasengschwandtner F: Lipodissolve for subcutaneous fat reduction and skin retraction. Aesthet Surg J 25:530-43, 2005
8. El-Ali K, Slator R, Solanki G, Hockley A, Nishikawa H: Multi-disciplinary management of spinal lipomas. J Plast Reconstr Aesthet Surg 62:964-8, 2009
9. Esko JD, Nishijima M, Raetz CR: Animal cells dependent on exogenous phosphatidylcholine for membranebiogenesis. Proc Natl Acad Sci USA 79:1698-702, 1982
10. Fleming KL, Davidson L, Gonzalez-Gomez I, McComb JG: Nondysraphic pediatric intramedullary spinal cord lipomas: report of 5 cases. J Neurosurg Pediatr 5:172-8, 2010
11. Gupta A, Lobocki C, Singh S, Robertson M, Akadiri OA, Malhotra G, Jackson IT: Actions and efficacy of phosphatidyl choline formulation and isolated sodium deoxycholate for different cell types. Aesthetic PlastSurg 33:346-52, 2009
12. Kroupa J: The role of pharmacology in the comprehensive prophylaxis of post-traumatic fat embolism. ActaChirOrthopTraumatolCech 60:114-22, 1993
13. Mahmud K, Crutchfield CE.: Lipodissolve for body sculpting: safety, effectiveness, and patient satisfaction. J Clin Aesthet Dermatol 5:16-9, 2012
14. Massie JB, Schimizzi AL, Huang B, Kim CW, Garfin SR, Akeson WH: Topical high molecular weight hyaluronan reduces radicular pain post laminectomy in a rat model. Spine J 5:494-502, 2005
15. Muthusubramanian V, Pande A, Vasudevan MC, Ramamurthi R: Concomitant cervical and lumbar intradural intramedullary lipoma. SurgNeurol 69:314-7, 2008
16. Naim-Ur-Rahman, Salih MA, Jamjoom AH, Jamjoom ZA:Congenital intramedullary lipoma of the dorsocervical spinal cord with intracranial extension: case report. Neurosurgery 34:1081-4, 1994
17. Noh Y, Heo CY: The effect of phosphatidylcholine and deoxycholate compound injections to the localized adipose tissue: an experimental study with a murine model. Arch PlastSurg39:452-6, 2012
18. Peter JC: Occult dysraphism of the spine: A retrospective analysis of 88 operative cases, 1979-1989. S Afr Med J 81:351-4, 1992
19. Reeds DN, Mohammed BS, Klein S, Boswell CB, Young VL:Metabolic and structural effects of phosphatidylcholine and deoxycholate injections on subcutaneous fat: a randomized, controlled trial. AesthetSurg J 33:400-8, 2013
20. RittesPG, Rittes JC, CarrielAmary MF: Injection of phosphatidylcholine in fat tissue: experimental study of local action in rabbits. Aesthetic PlastSurg 30:474-8, 2006
21. Salam GA: Lipoma excision. Am Fam Physician 65:901-4, 2002
22. Schimizzi AL, Massie JB, Murphy M, Perry A, Kim CW, Garfin SR, Akeson WH: High-molecular-weight hyaluronan inhibits macrophage proliferation and cytokine release in the early wound of a preclinical postlaminectomy rat model. Spine J 6:550-6, 2006
23. Schuller-Petrovic S, Wölkart G, Höfler G, Neuhold N, Freisinger F, Brunner F:Tissue-toxic effects of phosphatidylcholine/ deoxycholate after subcutaneous injection for fat dissolution in rats and a human volunteer. DermatolSurg 34:529-43, 2008
24. Skarjune R, Oldfield E: Physical studies of cell surface and cell membrane structure. Deuterium nuclear magnetic resonance studies of N-palmitoylglucosylceramide (cerebroside) head group structure. Biochemistry 21:3154-60, 1982

25. Sugata K, Kamimura S, Shijo H: Differential effects between tauroursodeoxycholic and taurochenodeoxycholic acids in hepatic fibrosis: an assessment by primary cultured Ito and Kupffer cells from the rat liver. J GastroenterolHepatol 11:451-8, 1996

26. YagimaOdo ME, Cucé LC, Odo LM, Natrielli A: Action of sodium deoxycholate on subcutaneous human tissue: local and systemic effects. DermatolSurg 33:178-89, 2007

Address reprint request to:

Emad Yosry, MD
Email: dr_emad_yosry@yahoo.com
Orthopedic department, Alexandria University, Alexandria, Egypt

الملخص العربي

تأثير مادة الفوسفاتيديل كولين على الأنسجة العصبية، دراسة نسيجية مرضية

البيانات الخلفية: يستخدم الفوسفاتيديل كولين ديوكسيكولات الان على نطاق واسع في اذابة الشحوم الحقني وغالبا ما يكون لأغراض تجميلية ومحدثا إذابة شحمية للدهون تحت الجلد وذلك يساعد على تعديل شكل الجسم وأيضا لتقليص الاورام الشحمية الصغيرة تحت الجلد. يقوم فريق البحث في هذا العمل بدراسة تأثير مادة الفوسفاتيديل كولين المذابة في الديوكسيكولات على النسيج العصبي لفئران التجارب لتقييم المخاطر من استخدام اذابة الشحوم الحقني كبديل للجراحة في علاج الاورام الشحمية بالعمود الفقري.

الغرض: بيان التأثيرالهستوباثولوجي لمادة الفوسفاتيديل كولين على النسيج العصبي لنموذج الفأر وذلك لبيان آمنيته في حال استخدامه للحقن الموضعي كبديل للجراحة في علاج الأورام الشحمية بالعمود الفقري.

تصميم الدراسة: دراسة هستوباثولوجية بأثر تقدمي على نموذج الفأر

الأساليب: ١٢ انثى فأر من فصيلة ويستر (العمر شهران) تم تقسيمها لمجموعتين بالتساوي وحقن مجموعة بمادة الفوسفاتيديل كولين والأخرى بمحلول ملحي ،وتم الحقن الموضعي خلال الجلد بالضفيرة الفخذية يوميا لأربعة أيام متتالية وتم تجميع العينات من الضفيرة الفخذية في اليوم الرابع ودراستها بالمجهرالضوئي. وتم قياس التأثير الباثولوجي بطريقة نصف كمية بتقسيمها لأربعة جوانب: الالتهاب، النخر ،التليف والتلف العصبي.

النتائج: الحقن المتكرر للفوسفاتيديل كولين ديوكسيكولات أدى الى التهاب حاد مجاورا للعصب مما أدى لتلف للأنسجة العصبية فضلا عن وجود ترسيب من ألياف الكولاجين الذي يعتبر كعلامة مبكرة على حدوث تليف ونخر بالأنسجة.

الخلاصة: في ضوء النتائج فإنه يتبين خطورة استخدام المادة قيد الدراسة بجوار الأعصاب ولا سيما بالأورام الشحمية بالعمود الفقري حيث تسبب التهاب و نخر شديدين.