Investigation of the Short-Term Effect of Chemonucleolysis with Chondroitinase ABC

Kazuta YAMADA1†, Shinobu TANABE1, Hiroshi UENO1, Akemi OINUMA2, Toyomi TAKAHASHI2, Satoshi MIYAIUCHI3, Satoshi SHIGENO1, Tsuneo HIROSE1, Kazuro MIYAHARA1 and Motoyoshi SATO1

1Department of Veterinary Clinical Radiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, 2Tokyo Research Institute, Seikagaku Corporation, Tokyo 207-0021, 3Obihiro Neuro-Surgery Hospital, Obihiro 080-0023, 4Research Institute of Information Science Development on Animal Medicine, Obihiro 080-0028 and 5Veterinary Teaching Hospital, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan

(Received 6 September 2000/Accepted 29 January 2001)

ABSTRACT. Chondroitinase ABC (C-ABC) is expected to be a novel agent for chemonucleolysis. The effect of C-ABC was investigated by magnetic resonance (MR) and radiograph. C-ABC was administered into the lumbar intervertebral disks on the clinically normal beagles (n=5), in a dose of 50 μl (12.5 units as C-ABC). MR scans were performed pre-dose, and 1, 3, 7, 14 and 28 days after administration of C-ABC, and the signal intensity (SI) of the nucleus pulposus was measured. Radiographs were taken pre-dose, and 1, 2, 3, 4, 5, 6, 7, 14 and 28 days post-dose, to evaluate narrowing of the disk space in terms of height index (HI). In addition, the quantity of the chondroitin sulfate (CS) and the hyaluronic acid (HA) in the nucleus pulposus were measured by high performance liquid chromatography on day 28 after dosing. SI and HI continuously decreased, following the injection to 37.1% and 78.9% of the pre-dose values, respectively. Statistically significant differences (p<0.01) were observed between the C-ABC group and the control group in the respects on day 1 post-dose. CS and HA contents of the nucleus pulposus were noted to be significantly decreased on day 28 (p<0.01) in the treated group. This agent proved to degenerate proteoglycans in the nucleus pulposus, thus progressively reducing the interdiskal pressure from day 1 post-dose onwards. It is concluded that C-ABC is expected to afford its efficacy from early in the course of chemonucleolysis.

KEY WORDS: canine, chemonucleolysis, chondroitinase ABC (C-ABC), height index (HI), magnetic resonance (MR).


Intervertebral disk hernia produces neurological deficits, caused by compression to the spinal cord or nerve roots via degeneration of the nucleus pulposus in the intervertebral disk, which is frequently seen in chondrodystrophy cases. Pharmacotherapy using corticosteroid or surgical intervention like hemilaminectomy is often selected for the treatment. The former does not completely remove the compression while the latter is invasive for the animal. Recently, chemonucleolysis has been applied as a less invasive treatment. Since Smith et al. reported that the chemonucleolysis using chymopapain applied for the first time to an intervertebral hernia case [12], and it was shown to be effective in the clinical setting application as well as in veterinary medicine [1, 10]. As the use of the proteolytic enzyme chymopapain has been reported to cause the side effects including neurotoxicity, anaphylaxis, subarachnoid hemorrhage [2-5], therefore its safety for this purpose remains questionable. Chondroitinase ABC (C-ABC) is expected as an alternative agent for chemonucleolysis. It specifically degrades some glycosaminoglycans such as chondroitin sulfate (CS) and the hyaluronic acid (HA). However, previous reports implied that C-ABC underwent morphological changes over an extended period post-dose [9, 13], our clinical experience with this therapy indicates that clinical manifestations improve in no few cases within one week after administration of C-ABC. This study was conducted to investigate short-term effect of chemonucleolysis with C-ABC by magnetic resonance (MR) and radiograph.

MATERIALS AND METHODS

Agent for chemonucleolysis: Highly purified pharmaceutical grade C-ABC (Lot. No. BC039P01) was provided by Seikagaku Corporation (Tokyo, Japan) as a mixture with non-ionic iodine contrast agent iohexol (Omnipaque® 350, Daiichi Pharmaceutical., Tokyo, Japan) in screw-capped vials (250 units/ml) per vial and was frozen at –18°C during transport and storage. The C-ABC mixture was thawed just before using. The results of a stability test of the C-ABC mixture revealed that it remains stable longer than 9 months when stored at –18°C [14]. A phosphate buffer-ihexol mixture was used as the control. Of four consecutive disks (L1-2, L2-3, L3-4 and L4-5), two intervertebral disks were injected with C-ABC and the two other disks with the control.

Procedure for chemonucleolysis: Five clinically normal beagles weighing from 9.0 to 13.0 kg (male, 1-year old) were used in this study. Animals were anesthetized by intravenous injection with thiopental sodium (Ravonal®, Tanabe Pharmaceutical, Osaka, Japan) and maintained by halothane (Fluothane®, Takeda Chemical Industries, Osaka, Japan) inhalation. The animal was then shaved and disinfected, and placed on an X-ray penetrative animal-holding device, which was inclined at 25 degrees to avoid damaging by a
spinal needle or contrast with the transverse process of the spinal nerve roots. A 70 mm, 23-gauge spinal needle with stylet was inserted into the disk under fluoroscopic control (MMS-155, Hitachi Medical Co., Tokyo, Japan). When the tip of spinal needle reached the anulus fibrosus of the disk, the stylet was pulled out, and a 31-gauge inner needle, which was 7 mm longer than the stylet, was inserted into the disk itself through the 23-gauge needle [1, 6, 10]. A microsyringe was used to inject 50 µl of 12.5 units of C-ABC into the disk. The administration dose is designed by previous report [14]. Radiographs were taken just after the injection to confirm accuracy of the administration, by enhancement of the nucleus pulposus since both chemonucleolysis agent and the control contained iohexol.

**Evaluation of MR images:** MR images were obtained with a 1.5 tesla magnetic field strength MR unit (VISART™, Toshiba, Tokyo, Japan). The imaging sequence used was spin echo (TR/TE=2,000/80 msec), producing T2 weighted images. The slice thickness of each section was 1.5 mm. The scan time of this image was 8 min 32 sec. Animals were placed under anesthesia with thiopental sodium during MR scan. MR scan was performed prior to and at 1, 3, 7, 14 and 28 days after administration of C-ABC. The signal intensity (SI) on region of interest on the nucleus pulposus was measured with a software within the MR unit. The ratio of the SI for non-injected T13-L1 to that at injection site was calculated, and evaluated in terms of percentage to the pre-administration value.

**Evaluation of radiographs:** The lateral radiographs were taken with X-ray film (HR-3, Fuji Film, Kanagawa, Japan) and an intensifying screen (HR-S, Fuji Film, Kanagawa, Japan). Images were obtained without anesthesia. The X-ray examination was performed pre-dose and at 1, 2, 3, 4, 5, 6, 7, 14 and 28 days after administration of C-ABC; thereby the narrowing of the disk space was evaluated in terms of height index (HI) [6], and expressed as percent of pre-administration value.

**Biochemical analysis:** The nucleus pulposus of L1-2, L2-3, L3-4 and L4-5 disks were sampled on day 28 after administration of C-ABC, the samples were analyzed for CS and HA by high performance liquid chromatography [15].

**Statistical analysis:** MR images, radiographs and biochemical analysis data were assessed for statistical significant differences using the Welch t-test.

**RESULTS**

**Evaluation of MR images:** Representative MR images after administration of C-ABC are shown in Fig. 1, and changes in SI of the nucleus pulposus observed (5 animals, n=10) are presented in Fig. 2. The SI of the nucleus pulposus from animals of the administration group was found decreased on day 1 post-dose, and thereafter continued to decrease to 37.1% of the pre-dose value on day 28. There were statistically significant differences (p<0.01) in SI between the C-ABC group and the control group from day 1 onwards.

![Fig. 1. Representative magnetic resonance (MR) images (spin echo pulse sequence, TR/TE=2,000/80 msec, 1.5 mm slice) of the canine lumbar spine after injection of chondroitinase ABC (C-ABC). C-ABC injected into the nucleus pulposus of L1-2 and L2-3 (arrows), and the control injected into that of L3-4 and L4-5. (a: pre-dose; b: 1; c: 3; d: 7; e: 14; f: 28 days after administration)](image-url)

**Evaluation of radiographs:** Representative radiographs taken after administration of C-ABC are shown in Fig. 3,
and changes in HI of the lateral view observed (5 animals, n=10) are presented in Fig. 4. The HI in the administration group was noted to be decreased on day 1, and continued to decrease thereafter to 77.9% of the pre-dose value on day 28. The statistically significant differences in HI were noted (p<0.01) between the C-ABC group and the control group throughout the observation period.

Biochemical analysis: The CS and HA contents of the nucleus pulposus after administration of C-ABC on day 28 post-dose are shown in Table 1. The quantity of CS is 8,602.5 nmol and 135.4 nmol in the control group and the treated group, respectively. The quantity of HA was 164.8 nmol and 20.7 nmol in the control group and the treated group, respectively. The nucleus pulposus CS and HA contents in the treated group were significantly lower (p<0.01) as compared to the control group.

During the entire period of this study, all animals remained free of changes in health status.

DISCUSSION

We studied the method of imaging evaluation for chemonucleolysis, prior to investigating the effect of C-ABC. At first, MR scanning parameters were settled. It is a well known fact that the contrast of MR image depends on pulse sequence, even if the image is what we call T2 weighted image, and shows subtle changes with pulse sequence. Spin echo pulse sequence, fast spin echo pulse sequence and gradient echo pulse sequence were compared, and the spin echo was selected because it has higher signal intensity of nucleus pulposus and less artifact. Secondly, we compared HI values of the lateral and ventro-dorsal views to seek an appropriate method of evaluation for radiographs. Although the HI decreased in both lateral and ventro-dorsal directions, the variation of ventro-dorsal direction was higher than that of lateral direction. It was possible to render the lumbar spine parallel to X-ray film by means of a pad in making lateral projection, but it was hard to keep the lumbar spine parallel in ventro-dorsal view because of the lumbar curvature. So, the lateral HI measurement was used for radiograph
Kudo et al. reported that the effect of chemonucleolysis delayed with aging. The nucleus pulposus undergoes either chondroid or fibrous change with aging [6], so that the present results might be different from previous report [9]. This needs further investigation in the near future.

In this study, continuously decreasing SI on the MR images and HI on the radiographs were observed. On 28 day post-dose, the progressive decrease in CS and HA contents of the nucleus pulposus was confirmed by the biochemical analysis. C-ABC produced degeneration of proteoglycan in the nucleus pulposus, thereby reducing the intradiscal pressure from day 1 post-dose onwards [11]. It is concluded that C-ABC is an alternative agent which is expected to afford the therapeutic effect chemonucleolysis within a short period after dosing.

REFERENCES


15. Toshida, K., Miyauchi, S., Kikuchi, H., Tawada, A. and Toku-
yasu, K. 1989. Analysis of unsaturated disaccharides from gly-
cosaminoglycan by high-performance liquid chromatography. 
うサギ冠状動脈におけるカプサイシンの弛緩作用——Yeon D.¹ Ⅲ Kwon S.¹ Ⅲ Lee Y.¹ Ⅲ Leem J.² Ⅲ Nam T.² Ⅲ Ahn D.² Ⅲ (¹ Department of Physiology, Yonsei University College of Medicine, Korea) 499-503

ウサギ冠状動脈のカプサイシンによる弛緩の機序を明らかにするために、収縮と細胞内Ca量を測定した。カプサイシン(1μMから30μM)は、プロスタグランジンPGF₂αによる収縮を濃度依存性に弛緩させ、細胞内Ca²⁺の増加を抑制された。カプサイシンの効果は洗浄により消失した。カプサイシンの効果はvaniloid受容体抗葉capsazepine(1μM)、非選択性陽イオンチャネル拮抗薬ルテニウムレッド(1μM)では影響を受けなかった。高濃度カプサイシン(100μM)前処置あるいはカプサイシンの類同投与により、カプサイシンの弛緩効果は減弱しなかった。外液K⁺濃度を80mMまで増加すると、カプサイシンの収縮及び細胞内Ca²⁺に対する効果は大幅に減弱した。Ca²⁺活性化K⁺チャネル阻害薬であるibetoxin(100nM)はカプサイシンの効果にはほとんどの影響であった。しかし、遅延性動性K⁺チャネル阻害剤である4-aminopyridine(4-AP, 1mM)はカプサイシンの収縮及び細胞内Ca²⁺に対する効果を大幅に減弱し、これらの成績から、ウサギ冠状動脈におけるカプサイシンは平滑筋に直接作用してこれを弛緩させること、そして、この弛緩は4-AP感受性遅延性動性K⁺チャネルの活性化によるものであることが示唆された。

公衆衛生学：
豚におけるTetracycline抗生素等の組織残留に対する酵素免疫法による生体検査技術——李厚長・李文華・李恒絃・趙明行²(大韓民国国立Seoul大学校獣医療系生理学教室、¹薬理学教室、²毒性学教室) 553-556
治療の用量として3種のTetracycline系抗生素剤等を別々に15頭の豚に投与し、投与前と休薬期間中に血液を採取し、血漿中のTetracycline系抗生素剤の濃度をsemi-quantitative酵素免疫法を使って測定し、内部標準液濃度値(10ppb)で比較した。サンプルと内部標準液濃度値との比率(B/Bs)の値を豚の組織に残留在する薬物の評価基準として、陽性と陰性を判定した。B/Bs比率値が1以下を陰性、1以上を陽性と判定した。採取をしなかった45頭の豚からの血漿サンプルは、Tetracycline系抗生素剤に対する陽性は見られなかった。Tetracycline系抗生素剤を投与した群において、Oxytetracyclineは休薬後8日まで血漿から薬物が検出された。またChlortetracyclineは休薬後4日まで、Tetracyclineは休薬後3日まで検出された。以上のことから、開発した酵素免疫法を使用した生体検査法は、と畜の豚においてTetracycline系抗生素剤の残留を容易に予測できる方法と考えられる。

外科学：
Chondroitinase ABCを用いた糖核分解疾患の投与後早期の効果の検討——山田一孝¹ 田辺忍¹・上野博史¹・生沼明美²・高橋齋²・宮内聡²・茂野智³・広瀬恒夫⁴・宮原和彦⁵佐藤基佳¹(¹帯広畜産大学獣医臨床放線病学講座、²生化学工業(株)、³帯広郡神経外科、⁴動物医学情報科学開発研究所、⁵帯広畜産大学附属家畜病院) 521-525
糖核分解疾患に効果が期待されているChondroitinaseABC(C-ABC)の効果をmagneticresonance(MR)およびX線像により検討した。臨床上健康なビーグル犬(n=5)の腹腔間板にC-ABCを50μgを12.5単位)投与した。MR像は、撮影日を投与前、投与後、1,3,7,14および28日後とし、糖核の信号強度(SI)を測定した。X線像は、撮影日を投与前、投与後、1,2,3,4,5,6,7,14および28日後とし、椎間の狭小化はheightindex(HI)により評価した。また、投与28日後に、糖核のコントロイシン硫酸(CS)およびヒアルロン酸(HA)含有量を高速液体クロマトグラフィーにより測定した。C-ABCを投与した椎間のSIおよびHIは経時的に低下し、投与28日後には投与前値に対して、それぞれ37.1%および78.9%となった。C-ABC投与群のSIおよびHIは、コントロール群と比較して投与1日後から危険率1%未満で有意差が認められた。
た、投与28日後の膿核のCSおよびHA含有量は、有意に減少していた（p<0.01）。C-ABCは、投与1日後から、膿核の変化および膿核内压の低下を認めたことから、膿核融解療法において投与後早期から効果を示すことが期待される。

馬のセボフランおよびイソフラン維持麻酔期における心血管系の経時的変化——山中隆史1・奥井寿臣1・小山秀一2・水野寛香1(1)日本中央競馬会美浦トレーニングセンター競走馬診療所、2)日本獣医畜産大学獣医学門内科学講座)

527-532

馬におけるセボフラン（S群）の臨床応用への基礎データを得る目的で、麻酔麻酔期（180分、12MAC）における心血管系指標の経時的変化をイソフラン（I群）と比較し、心拍数は両群共に概ね30 beats/minを示し、群間および経時的変化は認められなかった。肝機能、平均動脈圧（MAP）は、両群ともに有意な経時的変化を示したが、群間には有意差は認められなかった。心係数（CI）は、S群では経時的変化を示さなかったが、I群では経時的増加傾向を示し、群間には有意差が認められた。全身血管抵抗（SVR）は、両群共に有意な経時的増加が認められたが、S群の増加程度はI群を上回り、群間には有意差が認められた。心収縮力指標であるPEP/ETと群間の有意差は認められなかったが、I群では排出時間（ET）の延長により経時的低下を示した。以上のことから、I群MAPの増加はSVRの増加によるものだけでなく、PEP/ETの低下、すなわち心収縮力増加に伴う1回拍出量の増加をも反映している一方で、S群MAPの経時的増加はSVRの増加を反映し、CIの増加を伴わないことが明らかとなった。

PDA閉塞のために血管塞栓用コイルを追加留置したビーグル犬の1例（短報）——田中純1・永島由紀子1・星克一郎1・山根義久1(1)東京農工大学農学部獣医学科家畜外科学教室)

557-559

動脈管閉塞症（PDA）と診断した収縮期性高血圧を示すビーグル犬に血管塞栓用コイルによる閉塞法を実施した。術後3か月を経過した時点で残存血流が認められたため、コイルを追加留置した。従来、われわれのPDA症例では1個のコイルの使用で時間経過とともに閉塞が確認された。しかし、本症例では入手できる最大のコイル（8mm、5巻）を使用したが、術後3か月経過しても十分な閉塞が得られず、さらに1個の追加コイル挿入により完全閉塞を得た。

臨床繁殖学：

共培養および非共培養により作出された体外受精胚移植による黒毛和種産生子牛の生時体重と妊娠期間——沼辺孝1・及川俊孝1・菊地武1・堀内俊孝1(1)宮城県畜産試験場、2)広島県立大学)

515-519

体外受精胚移植により生産された子牛の生時体重と妊娠期間について、生体回収胚移植により生産され子牛と比較検討した。体外受精胚の作出について、発生培養法を2タイプにより行った。1)IVP-Co：培養液として5％子牛血清を添加したTCM199を用い、卵黄細胞と共培養を行った。2)IVP-NON-Co：培養液として鞘磷脂3日目まで5mg/mlのBSAを添加したCR1aa、それ以降は5％子牛血清を添加したCR1aaにより、いずれも8日間非共培養とした。体外受精に用いた卵子と生体回収の供試牛は黒毛和種を用い、精子は1頭の黒毛和種の凍結解凍精子を使用した。囊胚胚から胚盤期胚をホルスタイン種に発情期7±1日で移植を行い、単子のみのデータを用いた。IVP-CoとIVP-NON-Coによる子牛の生時体重は31.0kgと31.8kg、妊娠期間は291日と291日であり、いずれも差は認められなかった。生体回収胚移植による子牛の生時体重は27.2kg、妊娠期間は283.6日であり、体外受精後生産子牛の間に有意な差が認められた（P<0.01）。IVP-CoとIVP-NON-Coにより作出された黒毛和種体外受精胚移植による生産子牛は、生時体重と妊娠期間において差はないが、生体回収由来よりも生時体重は重く、妊娠期間は長いことが明らかになった。

耳由来遠藤細胞から再構築された胚に由来するフリーマーチンの発生（短報）——Hwang, W.-S.1・Cho, J.-K.1・Kim, K.-Y.1・Shin, S.-J.1・Kim, S.K.1・Park, J.-J.1・