Intraoral MR Lymphography : A New Method for Selective Enhanced Detection of the Cervical Lymph Nodes

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MR imagingにおける 経口性選択的頸部リンパ節造影

夏　廷毅　奥畑　好孝
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鉄コロイド製剤の経口投与によるMR画像における選択的頸部リンパ節造影を家兎10羽を用いて検討した．鉄コロイド製剤であるシデフェロン40mgFe（2ml）を市販の手動式鼻腔内噴霧器で30分間に6回に分けて口腔内に噴霧した．頸部リンパ節のMR画像を，両側の顔面静脈合流部にある後下顎リンパ節を中心として，噴霧開始前および開始後2時間に撮像を行い，組織標本を採取した．結果として，造影後のT2強調スピンエコー（SE）画像およびグラディエントエコー画像では，明らかな信号強度の低下を認め，プロトン密度強調SE画像では若干の信号強度の低下を認めたが，T1強調SE画像では信号強度の変化を認めなかった．鉄染色標本で同リンパ節のリンパ洞内に鉄剤の存在を確認した．同時に採取した扁桃にも鉄剤の存在を認めたが，膝下リンパ節および縦隔リンパ節には認めなかった．これより，鉄コロイド粒子が口腔内投与により扁桃の陰窩を介してリンパ流によりその領域リンパ節である頸部リンパ節に到達し，造影能を発揮したと考えられた．本研究ははじめて経口性頸部リンパ節造影の可能性を実証した．

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Introduction

Assessment of the status of the cervical lymph nodes is important for planning the treatment and determining prognosis in patients with malignancy of the head and neck regions. Almost all noninvasive diagnosing modalities including MR imaging depend mainly on measurement of nodal dimensions for assessing metastatic involvement[1)-5)]. Lymph node size is not however a reliable criterion because even normal sized nodes can have metastatic foci and enlarged nodes may be tumor free.

The use of superparamagnetic iron oxide (SPIO) particles in MR imaging has introduced a new class of contrast agents specific to the mononuclear phagocytosing system[6),7)]. SPIO particles effectively shorten T2, producing a signal loss in, for example, normal tissues of liver, spleen and lymph nodes[8)-10)]. MR lymphography, contrast enhanced imaging of lymph nodes, allows detection of the normal lymph nodes and could qualitatively aid in diagnosing their malignant nature[11)-14)]. Methods of delivering contrast agents to the lymph nodes via intralymphatical, interstitial or intravenous (IV) routes have been reported in animal experiments[11)-18)] and clinical studies[19),20)].

We have recently published results that confirm the use of inhalation MR lymphography as a new method for selective enhancement of the lung hilar and mediastinal lymph nodes[21)]. In performing an experiment, we incidentally found that the cervical lymph nodes also contained the contrast agent. We inferred from those findings that lymphographic MR contrast agents could reach the cervical lymph nodes by intraoral administration and had the potential of selective enhanced detection of the nodes on MR imaging. The aims of this study were to prove the potential of this method and investigate how intraorally administered contrast agents can reach the cervical lymph nodes in a healthy animal model.

Materials and Methods

Animals and Anesthesia : Ten Japanese white rabbits (Tokyo

Jikken Doubutsu, Tokyo, Japan) of both sexes and weighing 3.0-3.2 kg were used. For MR imaging, anesthesia was induced by intravenous injection of secobarbital (20 mg/kg, Ional®, Yoshitomi Seiyaku, Osaka, Japan) through the ear vein and intraperitoneal administration of urethane (0.75 g/kg, Sigma Chemical, St. Louis, MO), and was maintained by additional injection of secobarbital, as required.

Contrast Medium and Administration Technique : Cideferron (Ferricon®, Nihon Zoki Seiyaku, Osaka, Japan) was used. This substance is an iron colloid of dextrin and citrate iron (III) complex with a particle size of 50-80nm in diameter, which was previously described as a paramagnetic particulate, T2 shortening agent with T1/T2 relaxivities of 0.22/2.4 (mmol/l · sec)$^{-1}$ at 1.5 T and 22°C [16]. It results in a decrease in signal intensity in lymph nodes, as confirmed in animal [16), 21)] and clinical [19] studies. After obtaining precontrast imaging, the agent was sprayed into the oral cavity with a manual air compression nebulizer (Microflator, Fisons® Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) at doses of 40 mg Fe/2 ml. The administration was divided into six times in 30 minutes.

MR imaging : MR imaging was performed in a prone position with a whole-body MR system (Gyroscan S15HP, Philips, Netherlands) at a field strength of 1.5 T. Nine transverse multisection images of the cervical area were obtained with a field of view of 150 mm in width, 3 mm in slice thickness, 0.3 mm on interval and a 256×256 matrix. A circular type surface coil (diameter 8 cm) was used with a half-encoding method. T1-, T2-/proton density-weighted spin-echo (SE) images and (715/30/2, 2000/30, 60/2) and gradient-echo (GE) images 375/14.4/4°, 15° flip angle were obtain respectively, before and 2 hrs after administration of the agent.

Histological examination : Cervical lymph nodes (retromandibular, retroparotid and retropharyngeal nodes [22)]) and palatine tonsils, as well as mediastinal and popliteal nodes as a control, were obtained to verify the presence of stainable iron after the postcontrast MR imaging. Dissection was performed to correlate the morphology of the cervical lymph nodes with the appearance on MR images. Samples were fixed in 10% formalin, embedded in paraffin and stained for iron with Perls Prussian blue.

Results

MR images : Because the size of each lymph node was almost the same as the slice thickness and was typically visible only on a single slice, the section positions were chosen with care to ensure that an optimal section through the retromandibular nodes could be imaged with a minimum of partial volume artifacts. The retropharyngeal and retroparotid nodes were visualized in certain other sections, but not in all cases.

These lymph nodes which were 2-3 mm in diameter were identified on pre- and postcontrast images in animals. In precontrast MR images (Fig.1 (A), (C), (E) and (G)), the nodes were shown as hypointense compared with fatty tissues and nearly isointense with muscle tissues on T1-weighted and proton density-weighted SE images. On T2-weighted SE and GE images, the nodes had high signal intensity and were shown as hyperintense compared with muscle tissues. In postcontrast MR images (Fig.1 (B), (D), (F) and (H)), the retromandibular nodes showed a homogeneous decrease in signal intensity on T2-weighted SE and GE images. Comparison of the four sequences revealed significant signal loss on the T2-weighted SE and GE sequences, followed by the proton density-weighted SE sequence, and no changes on the T1-weighted SE sequence. No significant changes in the signal intensity of the retropharyngeal and retroparotid nodes could be visualized however on postcontrast images.

Histological examination : Histological examination showed that all nodes were in a normally active structure. Numerous follicles with germinal centers situated in the cortical area and plasma cells populated in the medulla. In the retromandibular nodes, stainable iron particles could be identified in macrophages throughout the marginal, intermediate and medullary sinuses (Fig.2). In the retroparotid lymph nodes, a focal enrichment of iron particles in macrophages of the medulla was noticed (Fig.3). The iron particles were also found in the tonsillar parenchyma (Fig.4). No iron particles were seen in the retropharyngeal, mediastinal or popliteal lymph nodes.

Discussion

The intraorally administered agent could be detected in the cervical lymph nodes, which suggests that some places must have permeability of particulates in the oral cavity. All epithelium of the oral cavity, except for the crypts of the tonsil, are covered with stratified squamous cells, which have closely packed microfolds on their surface and less permeability of particulates. The cryptal epithelium of the tonsil, particularly in the major part of the lacuna, is mainly lined by reticular epithelium or desquamation [23)]. Recent scanning electron microscopy studies have demonstrated the presence of micropores on the cryptal epithelia of palatine tonsils and used the term "microcrypts" in rabbits [24)] and humans [25)]. One of them, type I microcrypt, 5-15μm in diameter, is a widened intercellular space between epithelial cells and leads deep into the subepithelial lymphoid tissues [25)]. Through this tunnel-like passages, foreign substances can penetrate to the tonsillar parenchyma [23)]. In our histological studies the iron particles were found in the tonsils and cervical lymph nodes, but not in the other nodes such

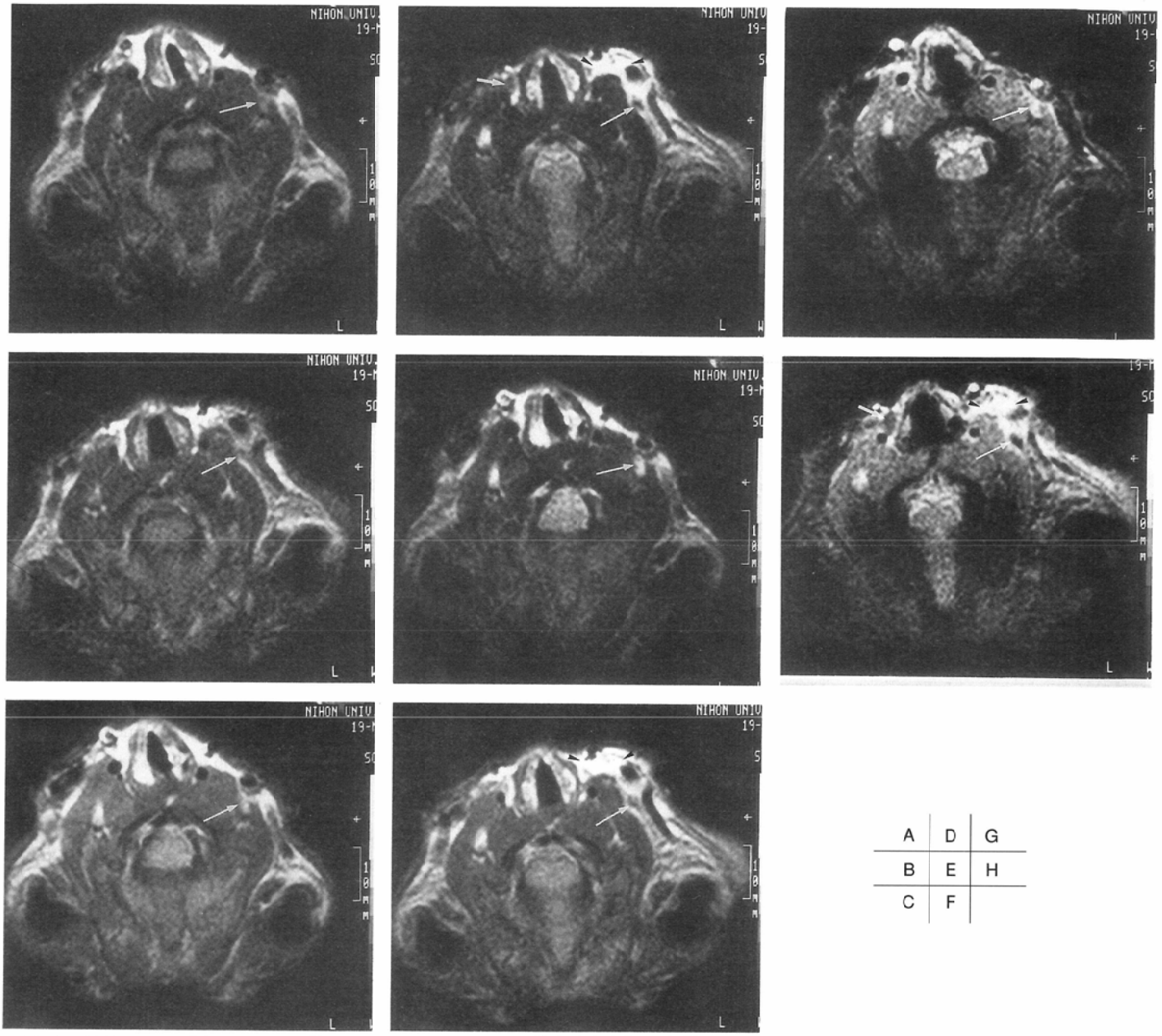

Fig.1 MR images of the retromandibular lymph nodes on pre-((A), (C), (E) and (G)) and postcontrast ((B), (D), (F) and (H)) enhancement. Precontrast, the node (long arrow) shows low signal intensity on the T1-weighted SE (A), moderate signal intensity on the proton density-weighted SE (C) and high signal intensity on the T2-weighted SE (E) and GE (G) images. Postcontrast, significant signal loss on the T2-weighted SE (F) and GE (H), moderate signal loss on the proton density weighted (D) and no signal changes on the T1-weighted SE (B) images are visualized (long arrow). A decrease in signal intensity is also visualized in another node (F and H, short arrow), which was identified in the next slice on the precontrast images (not provided). A high signal intensity area in the left subcutaneous tissues on the postcontrast images ((D), (F) and (H), arrow head) is local edema, which was a side effect due to venous injection through the ear for anesthesia.

as mediastinal or popliteal nodes. These results also proved the cryptal epithelial permeability of particulates.

Because the particles are too large to pass through capillary walls[26], it was thought that the agent within the tonsillar parenchyma might be transported to the regional lymph nodes by draining lymphatics or partially engulfed by macrophages in the tonsil. And because the histological studies showed that the iron particles in the tonsil were much lower than those in the cervical nodes, transportation through lymphatic routes rather than the phagocytosis of tonsillar macrophages seemed to be the dominant process of clearance.

An intraorally administered agent allows free entrance to tonsillar crypts, but its ratio is limited because most of it is swallowed together with saliva and transported to the gastrointestinal tract. Iron can be absorbed in ferrous ions but not in the ferric state[27]. Superparamagnetic iron oxide and ferric ammonium citrate are used as oral gastrointestinal contrast agents, and do not cause clinical side effects at doses of 400 mg Fe[28] and 600-1200 mg Fe[29], respectively. So despite the comparatively large dosage, intraoral administration of ferric

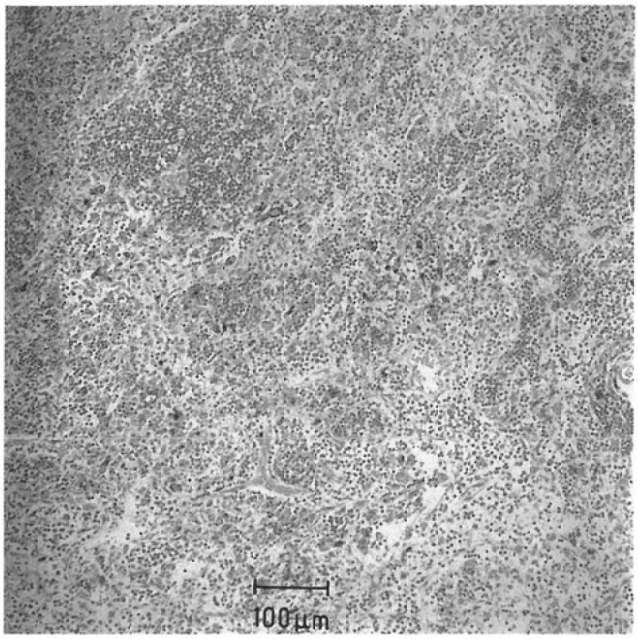

Fig.2 Photomicrograph of histologic specimen of the retromandibular lymph nodes. Stained iron particles as black spots are diffusely distributed throughout the medullary sinuses.

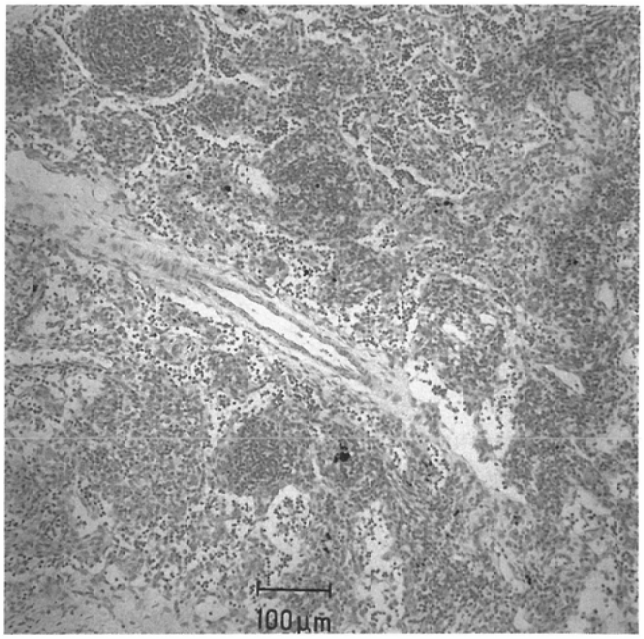

Fig.3 Photomicrograph of histologic specimen of the retroparotid lymph nodes. Stained iron particles are located with a feature of focal enrichment in the medullary sinuses.

iron, whether paramagnetic or superparamagnetic, is considered to be safe.

In the intraoral method, only a small quantity of the agent can reach the cervical nodes, while a large quantity is excreted. This means that the agent actually absorbed in the body is minimal but can be effectively used for contrast enhancement of the cervical nodes. Through IV administration, agents can reach all lymph nodes of the body and may be of particular importance for clinical use[15), 17), 18), 20)]. However, with this method, agents accumulate not only in all nodes but also in the liver, spleen and bone marrow, which are unnecessary for lymphography. Furthermore, the actual clinical diagnosis of metastatic lymph nodes is almost entirely limited in the regions of interest. Therefore, intraoral administration is considered to be a more efficient technique for contrast enhancement of the cervical nodes than the IV method. This method, which is noninvasive and simple, also has an advantage over the intralymphatical and interstitial methods, which require a local surgical maneuver or multiple interstitial injections.

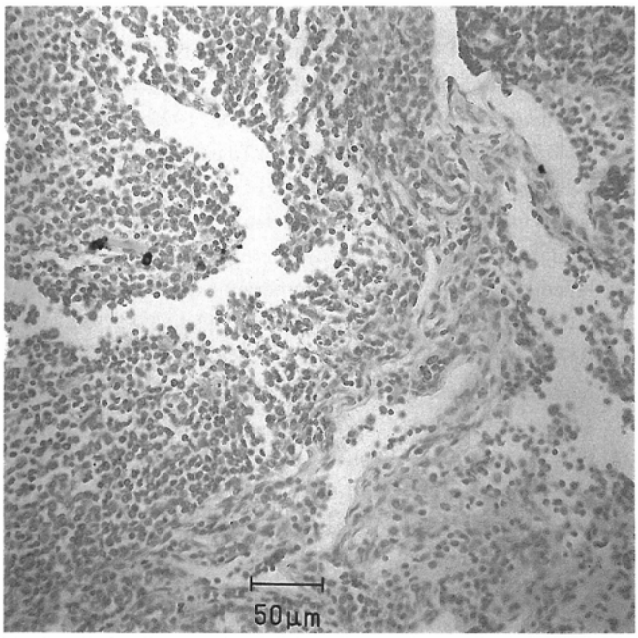

Fig.4 Photomicrograph of histologic specimen of normal palatine tonsil. Aggregated iron particles are found in the tonsillar parenchyma.

Although the agent used in this study was paramagnetic and more than 30 times weaker than SPIO in T2 relaxivity, it led to adequate signal reduction throughout the retromandibular nodes. This suggests that this method has sufficient potential to deliver the agents to the cervical nodes and the administered dose can be reduced through the use of SPIO.

The three nodal groups in the cervix showed differences in contrast enhancement on MR images and in accumulation of iron particles in histological studies. These results are consistent with the fact that this method is based on lymph draining from the tonsils to their regional nodes. Because of the limitations of the lymph draining routes, not all cervical nodes may be enhanced to the same degree. This would seem to be a disadvantage when a survey of all cervical nodes is attempted. However, this method could physiologically assess regional nodal involvement for oropharyngeal malignancies such as tonsillar lymphomas or carcinomas, because metastases to regional nodes generally spread through lymph draining routes. This was a preliminary study, and further studies are necessary to investigate the optimal doses, time-response relationships and choice of contrast agents, and to clarify whether this

method can be used for clinical diagnosis.

Conclusion

Our results revealed for the first time that intraorally administered contrast agents could be delivered to the cervical lymph nodes of the oropharyngeal region through the crypts of the tonsils. This experimental study of intraoral MR lymphography may serve as a tool for detecting cervical lymphnode analysis.

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