Meeting Report

Sheffield 2nd International Workshop on experimental studies of nephrogenic systemic fibrosis, 15th June 2012, Sheffield, UK

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Abstract: A group of experienced researchers in the field of experimental studies investigating the condition nephrogenic systemic fibrosis (NSF) were invited for a workshop to share knowledge, and consolidate the current understanding of the pathophysiology of this complication associated with the administration of gadolinium based contrast agents (GBCAs) in patients with advanced renal impairment. The workshop started with a session on the diagnostic criteria and epidemiology of NSF. This was followed by a session on in vitro studies using fibroblasts followed by a session on in vivo experimental studies employing different animal models. The workshop was finished with discussing the safety of using macrocyclic GBCAs in patients suffering from NSF.

Key Words: Nephrogenic systemic fibrosis (NSF); gadolinium based contrast agents (GBCAs); chronic kidney disease (CKD)

First session

Diagnostic criteria of NSF by Shawn Cowper, Yale University, USA

The diagnosis of NSF is not easy and can be confused both clinically and histologically with other diseases (e.g., scleromyxoedema, systemic sclerosis, morphea, lipodermatosclerosis, eosinophilic fasciitis). A group of eminent clinicians and dermatopathologists developed a user-friendly clinicopathological scoring system identifying major and minor clinical and histological criteria for the diagnosis of NSF. The scoring system was developed in parallel with workup recommendations and an image atlas to assist in the identification of salient clinical and histological points. The system culminates in a grid that stratifies cases into consistent, suggestive or inconsistent with NSF (1).

Epidemiology of NSF in the USA and regularity changes for GBCAs by Ali Abu-Alfa, Yale University, USA and American University of Beirut, Beirut, Lebanon

The prevalence of NSF in the USA since association between NSF and the use of GBCAs was suggested in 2006 was reviewed as well as the evolution and chronology of Food and Drug Administration (FDA) regulatory labeling changes for GBCAs. The problems in identifying patients with reduced renal function before the administration of GBCAs were also discussed. In addition, the impact of the regulatory changes on clinical practice and GBCA-enhanced imaging was reviewed. The FDA regulations and contraindicating the use of low stability GBCAs in patients with advanced renal impairment and robust local policies on the safe use of these agents have resulted in marked reduction in the prevalence of NSF in the USA. However, the presenter warned that new cases of NSF might continue to surface from areas without adequate regulatory infrastructure or awareness.

Epidemiology of NSF in Europe and European guidelines by Henrik Thomsen, University of Copenhagen, Copenhagen, Denmark

The history of the development of GBCAs and the toxicology of lanthanides were briefly reviewed. The
prevalence of NSF after the use of low stability GBCAs was presented and a high incidence of 18% of histologically proven NSF cases was found in one report of patients with chronic kidney disease (CKD) grade 5 (GFR less than 15 mL/min/1.73 m²) who have received the non-ionic linear GBCA gadodiamide (Omniscan, GE Healthcare, USA) (2). The presenter suggested that the uneven distribution of NSF in Europe may be due to a combination of lack of awareness and difficulty in diagnosing NSF as it mimics several skin lesions. He also indicated that the real prevalence of NSF is likely to be underestimated as many patients with this condition might have died undiagnosed. Nevertheless, it seems with the implementation of new recommendations by the European regulatory authorities which classified GBCAs into three groups; high risk [non-ionic linear GBCAs and the ionic linear chelate gadopentate dimeglumine (Magnevist, Bayer, Germany)], intermediate risk [all the other ionic linear GBCAs] and low risk [the macrocyclic GBCAs] and guidelines by professional bodies on safe use of GBCAs particularly those of the European Society of Urogenital Radiology (ESUR) have almost erased the disease.

Second session

In vitro studies of the effects of GBCAs on fibroblasts and organ cultured skin

The Glasgow data by Michael Edward, Glasgow University, Glasgow, UK

Experimental in vitro studies in Glasgow have made the following observations; fibroblasts from skin specimens of patients with NSF exhibit a myofibroblast phenotype and synthesize excess levels of hyaluronan and collagen compared to control fibroblasts. NSF patient serum stimulates control fibroblast hyaluronan and collagen synthesis. The low stability non-ionic linear GBCA gadodiamide is a potent stimulus of fibroblast cell proliferation. This effect was also observed with the stable macrocyclic agents but at much higher concentration by a factor of 50 in comparison to low stability agents (3). Gadodiamide stimulates a modest increase in control fibroblast hyaluronan synthesis but does not stimulate collagen synthesis. Control fibroblasts require only a brief exposure to gadodiamide to stimulate proliferation.

Microarray analysis suggests that gadodiamide-exposed fibroblasts synthesize less matrix molecules than control fibroblasts and do not adopt a fibrotic phenotype.

The Michigan data by James Varani, University of Michigan, USA

Extensive data from in vitro studies were presented. In organ-cultured normal skin gadodiamide induced alteration in skin collagen metabolism which includes procollagen synthesis is reduced, metalloproteinase 1 (MMP-1) and its tissue inhibitor (TIMP-1) are elevated but >90% of MMP-1 is complexed with TIMP-1 causing inactivation of the MMP leading to reduction in collagen fragmentation and increase in collagen deposition. Similar responses to gadodiamide were observed in organ-cultured skin from patients with end stage renal disease (ESRD). Gadodiamide also increased proliferation of fibroblasts and production of MMP-1, TIMP-1 and hyaluronan in cell culture. The effects of gadodiamide in cell culture of fibroblasts from skin of patients with ESRD were the same as fibroblasts from normal (4).

Recent experimental data demonstrated that fibroblasts exposure to gadodiamide activates intracellular signaling pathways essential for biological function but no direct activation of platelets derived growth factor (PDGF) receptor. Gadodiamide was found to interfere with CCN3 a member of a family of matricellular proteins (CCN) which includes CCN1, CCN2 and CCN3. The CCN proteins are also known as CTGF (Connective Tissue Growth Factor). They are involved in the regulation of various cellular functions, such as proliferation, differentiation, survival, adhesion, migration and in both internal and external cell signalling. CCN3 down-regulates mesangial cell proliferation driven by PDGF and CCN2 a mediator of transforming growth factor beta (TGF-β) which stimulates collagen production.

Gadolinium salts (phosphate and chloride) produced similar effects to gadodiamide by binding to fibroblasts and be phagocytosed triggering intracellular signaling pathways similar to those associated with gadodiamide with no evidence of growth factor receptor activation.

The Sheffield data by Sheila MacNeil, University of Sheffield, Sheffield, UK

The in vitro studies on human fibroblasts in culture showed that gadodiamide stimulates fibroblast proliferation and collagen production but no significant effect on keratinocytes. The stable macrocyclic agent meglumine gadoterate (Dotarem, Guerbet, France) had no effects except at very high (10 mM) concentrations (5).
Third session

In vivo studies investigating the pathophysiology of NSF

Bayer data by Hubertus Pietsch, Head of MR and CT Contrast Media Research, Bayer Healthcare, Berlin, Germany

Extensive data were produced by Bayer researchers since the recognition of possible association between NSF and GBCAs in 2006. Their results demonstrated that multiple dosing of low stability non-ionic linear GBCAs in rats with normal or reduced renal function (5/6 subtotal nephrectomy) induce skin lesions consistent with human NSF. This effect was not observed with other GBCAs (6). They also had shown long term retention of gadolinium in the skin of rats with normal or reduced renal function up to a year after the injection of non-ionic linear chelates but minimal retention with the macrocyclic agents. The amount of retained gadolinium in the skin was higher in rats with reduced renal function (6-8).

The intravenous administration of the non ionic linear chelate gadodiamide in the normal rat induced an increase in the production of cytokines, enzymes and growth factors which includes vascular endothelial growth factor (VEGF), osteopontin (OPN) and TIMP-1 (9).

Guerbet data, by Jean-Marc Idée and Nathalie Fretellier, Guerbet Research Department, Guerbet, Paris, France

In vivo experiments in rats with subtotal nephrectomy received multiple intravenous injection of GBCAs demonstrated gradual in vivo dissociation of the non-ionic linear GBCA gadodiamide while the ionic macrocyclic agent gadoterate remained stable (10). Further studies revealed that hyperphosphataemia hypersensitizes renally-impaired rats to gadodiamide induced fibrosis, confirming that hyperphosphataemia is a risk co-factor for NSF (11).

In a rat model of adenine induced renal impairment they demonstrated positive correlation between the extent of renal impairment and the severity of skin lesions and the amount of gadolinium that was retained in the skin. They also demonstrated that the ionic linear GBCA gadopentetate dimeglumine (Magnevist, Bayer, Germany) induces transient punctuate skin lesions which was not substantiated by histology in rats with severe adenine induced renal impairment. They also observed gradual in vivo dissociation, with release of soluble Gd\(^{3+}\) in rats receiving this agent.

Further studies in the adenine induced renal impairment model demonstrated that treatment with paclitaxel which interferes with TGF\(\beta\) biological pathway attenuates gadodiamide-induced fibrosis in rats with moderate renal impairment but no effect when the renal impairment was severe.

The Sheffield data by John Haylor, University of Sheffield, Sheffield, UK and Josef Schroeder, University Hospital Regensburg, Germany

In rats with subtotal nephrectomy gadodiamide produced a 40-fold greater increase in skin gadolinium than the ionic macrocyclic agent gadoterate. An electron dense filamentous material, detected within extracellular matrix, displayed a “halo” appearance, associated with collagen fibrils and electron-dense intracellular fragments of collagen fibrils within activated fibroblasts and in histiocytes. Energy-Filtered Transmission Electron Microscopy (EFTEM) scanning demonstrated that these electron-dense features are positive for gadolinium. No abnormalities were detected in the rat skin following the administration of gadoterate and no positive scanning for gadolinium was observed. Gadodiamide also increased dermal cell count, dermal thickness and collagen bundle density with enhanced immunostain for CD34, fibroblast specific protein 1,4-hydroxy-prolyl-hydroxylase and factor XIIIa. Circular staining for \(\alpha\)-smooth muscle actin indicated new blood vessel formation. Skin of rats receiving gadoterate remained unchanged.

In summary, this study showed the ultrastructural location of gadolinium retention in the skin which has been identified at two sites, extracellularly around segments of the collagen fibril and intracellularly to fragments of collagen fibrils within fibroblasts and also in histiocytes following the administration of a low stability non-ionic linear GBCA gadodiamide. The deposition of gadolinium in the skin around segments of the collagen fibril was associated with a ‘halo’ of an electron dense material which requires identification. Following the macrocyclic Gd chelate (gadoterate), skin features similar to nephrogenic systemic fibrosis were absent and no gadolinium was detected within skin collagen fibrils or fibroblasts. Of the tissues examined, the greatest difference in gadolinium retention between linear or macrocyclic gadolinium chelates was in the skin (12).

The final session

The use of macrocyclic gadolinium based contrast agents in patients with NSF: is it safe?

By Gertraud Heinz-Peer, Medical University of Vienna, Vienna, Austria

A case of a patient with NSF in whom the disease progressed
following administration of a macrocyclic agent for MR angiography prior to a second renal transplant operation was presented. Improvement of the NSF symptoms was observed following a successful second renal transplantation but the disease progressed again two years later with deterioration in renal function and the final outcome the patient became haemodialysis and wheelchair dependent. No GBCA was administered after the second renal transplant (13). It is not clear from this case whether the deterioration in the NSF manifestations was induced by the administration of a macrocyclic agent or caused by the progression of renal impairment due to failed renal transplantation. The general consensus of the discussion was the deterioration in renal function played a major role in the progression of the NSF especially after the second renal transplantation since no GBCA was used after the operation. The contribution of the macrocyclic agent to the deterioration of the NSF symptoms prior to the second operation when the patient had severe reduction in renal function was thought to be speculative. Nevertheless, it was agreed that the use of GBCAs in patients with NSF should be contraindicated and if it is deemed absolutely essential to undergo a contrast enhanced MRI examination the smallest possible dose of a macrocyclic agent should be used after consultation with a nephrologist.

Acknowledgements

I am most grateful to all the above mentioned speakers who shared their immense knowledge in their presentations with remarkable clarity. Their input in the discussion has given us a new insight into the complicated biological mechanisms that are involved in the pathogenesis of NSF.

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References