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Freezing Nitrogen Ethanol Composite (FNEC), a new effective cryoablation technology as an adjuvant modality treatment for giant cell tumor of bone

Po-Kuei Wu [1,2,3], email: drwuvgh@gmail.com

Cheng-Fong Chen [1,2,3], email: cf_chen@vghtpe.gov.tw

Jir-You Wang [1,2,3], email: yollywang@gmail.com

Wei-Ming Chen [1,2,3], email: wmchen@vghtpe.gov.tw

1. Orthopaedic Department School of Medicine, National Yang-Ming University
2. Department of Orthropaedics and Traumatology, Taipei Veterans General Hospital
3. Therapeutical and Research Center of Musculoskeletal Tumor, Taipei Veterans General Hospital

Background:

High incidence of local recurrence is a major concern for the treatment of giant cell tumor (GCT) of bone by surgical curettage. Adjuvant therapy by phenol can decrease local recurrence rate; however, the efficacy is limited by its low margin of cytotoxicity (0.2mm). Cryoablation is a safe, cost-effective and reliable adjuvant option to lower the recurrent rate. Liquid nitrogen (LN) is commonly introduced in cryosurgical procedures treating bone tumors. Cryoablation cycle that employs the procedures such as rapid freezing to -40~-60**°**C and successive slow thawing to promote massive ice crystallization and re-crystalliztion and cause cell death. However, the use of direct-pour of LN exposes the surrounding tissues to the risk of damaging spill.

Questions/Purposes:

A new Freezing Nitrogen Ethanol Composite (FNEC) was therefore developed as an effective adjuvant modality for ablation of bone tumor cells.

Patients and Method:

To ensure the effectiveness of cryogenic treatment, cryoradiation through 1-cm thickness of cortical bone was measured by the temperature shift of an FNEC-embedded tissue with an inserted thermal couple. Specimens of human giant cell tumor of bone were harvested by curettage surgery from a 35-year old male. The collected tumor tissues were divided into four groups, negative control (GCT without treatment), positive control (GCT was treated with liquid nitrogen for 5 minutes), FNEC group 1 (GCT was treated with FNEC for 30 seconds), and FNEC group 2 (GCT was treated with FNEC for 5 minutes). After cryolation was performed by embedding the specimens either with LN or FNEC and subsequently thawing, the effects of tumor-ablating were determined by histological microscopy,

TUNEL assay and western blotting method.

Results:

The solidified ethanol-based FNEC reached to the lowest temperature at -111.4°C within 40 seconds. Consistent data were demonstrated by experiments in triplicate. FNEC rapidly radiated the cryogenic effect through 1-cm cortical bone and the underneath temperature dropped to -60°C in 5 minutes. Results of histological microscopy revealed different characteristics among the four groups. In the group solely treated with LN (positive control), proliferating mononuclear stromal cells and random distribution of osteoclast-like cells as well as multinucleated giant cells were characterized. The cryoablation groups (positive group, FNEC group 1, and FNEC group 2) showed mononuclear cells and osteoclasts shrinkage, coagulative necrosis and fibrosis, indicating tumor cells death. The results of TUNEL assay suggested that apoptosis was significantly increased by cryogenic treatment in comparison with the negative control group. Comparable cryoablation effects were observed between the group treated with FNEC for 5 minutes and that solely treated with LN. Whereas, apoptotic rate was lower while FNEC was only applied for 30 seconds. Elevated expression of pro-apoptotic regulator Bax3 was found in all cryogenic treatment groups. In addition, lengthened FNEC treatment (5 minutes) was associated with a higher level of Bax3 in comparison with the other two groups treated with cryoablation. Likewise, increases in cleavage processing of caspase 8 and 9 in an apoptosis activation pathway were detected in both LN group and FNEC group 2. Changes in the levels of apoptosis regulators were consistent with the results of histological observation and TUNEL assay.

Conclusions:

The new FNEC technology as an adjuvant modality was effective for cryoablation in treating the GCT of bone. The FNEC in gelled phase was accessible to thoroughly fill the bone cavity during the surgery. It also improved LN-handling safety during the tumor cryoablation procedures. Analysis of changes in the levels of cleaved caspases 8 and 9, Bcl2 and Bax indicated that FNEC-embedding cycles for 5 minutes provided a comparable apoptosis-inducing effect for the group of LN only. In the perspective of safety and effectiveness for cryosurgical procedures, the FNEC technology deserves further development as a part of combined modality treatment on bone tumors.

Figure 1. Photography of Freezing Nitrogen Ethanol Composite (FNEC). FNEC could maintain solid to gelatinous phase and easily transport to the specific part of the bone cavity during the surgery

Figure 2. Histological microscopic characteristic of significant mononuclear cells and osteoclast shrinkage, coagulative necrosis and fibrosis indicated tumor cells death in group in Liquid nitrogen 5min, FNEC 30 sec and FNEC 5 min.