

Original Article

Nitrous oxide triggers synergistic effects of High Intensity Focused Ultrasound on tissue injury via reinforcing ultrasonic cavitation in tissues

Mingyuan Pan^{1*}, Fang Liu^{2*}, Zedan Tian¹, Xiaoyang Xu²

¹Department of Anesthesiology, The Second Hospital Affiliated to Chongqing Medical University, Chongqing 400016, P. R. China; ²Department of Obstetrics and Gynecology, The Second Hospital Affiliated to Chongqing Medical University, Chongqing 400016, P. R. China. *Equal contributors.

Received November 10, 2015; Accepted March 25, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: We find that various anesthetics play different influences on High Intensity Focused Ultrasound (HIFU). Especially for nitrous oxide (N₂O), its utilization plays a greater influence on treatment. We selected 98 patients with primary hepatic carcinoma, who planned to perform HIFU. These patients were divided into Anesthetic A group (N₂O + isoflurane + propofol + remifentanyl), Anesthetic B group (isoflurane + propofol + remifentanyl) and Anesthetic X group (propofol + remifentanyl) due to the used anesthetics. Red blood cells were observed in vitro and red blood cell morphology through transmission electron microscope, after pumping pure nitrous oxide in red blood cell suspension. Red blood cell suspension was divided into control group, nitrous oxide group, HIFU group and nitrous oxide combined HIFU group. After experimental treatment, red blood cell number and potassium concentration were obtained by virtue of blood routine examination and blood gas analysis. Results indicated that dermohemia and edema of operative sites in Group A were more significantly than additional two groups ($P < 0.05$). Experiment of red blood cells in vitro found that number of residual red blood cells in N₂O combined HIFU group was the least, and rising of potassium concentration was a little high. After two independent sample variance analyses, it had significant difference with other groups. In conclusion, application of nitrous oxide in HIFU operation aggravated injury degree of tissue. Combined utilization of nitrous oxide and HIFU played a synergistic effect on tissue injury and had mechanism that N₂O increases micro bleb nucleon in tissue to reinforce ultrasonic cavitation effect and produce a synergistic effect.

Keywords: High Intensity Focused Ultrasound, nitrous oxide, operation, synergistic effect

Introduction

In 1793 the nitrous oxide (N₂O) was firstly discovered [1, 2]. Humphrey Davy found that it had an analgesic effect in 1799 and it had over 160 years of history as an anesthetic [3]. Though reports concerning side effects of nitrous oxide are a great many [4], no tangible proofs can prove that applications of nitrous oxide have negative effects on the health of patients and medical workers in clinically. Applications of nitrous oxide are still feasible in clinical anesthesia.

When nitrous oxide anesthesia is applied to High Intensity Focused Ultrasound (HIFU) in clinical work [5, 6], body injury is aggravated. This study discusses the relation between nitrous oxide and HIFU effect preliminarily by

observing the degree of curing edema of patients with HIFU by using nitrous oxide and experiment of red blood cells in vitro.

Materials and methods

Recruitment of volunteers and experiment instrumentations

This study has already been approved by Ethics Committee of the hospital and has told patients and families, who signed informed consent. The study selected 98 of patients with primary hepatic carcinoma, who accepted HIFU operative treatment in Haifu Cancer Center of our hospital and had no restriction on gender and age. The demographic information and clinico-pathological data were listed in **Table 1**. Before ASA grade included I-II grades, routine inspec-

Nitrous oxide enhances synergistic effects

Table 1. Demographic information and clinicopathological features of 98 patients with hepatocellular carcinoma

Characteristics	All patients
Age (years, median, range)	43 (27-69)
Gender (male/female, %)	39/61
Tumor staging (TNM, n, %)	
I or II	35 (36%)
III or IV	63 (64%)
Tumor size (cm, median, range)	8 (4-14)
Tumor size >10 cm (n, %)	43 (44%)
Lymph node (n, %)	8 (8%)

tion had no obvious abnormality. Experiment of red blood cells in vitro selected autologous recovered whole blood 400 ml of a patient before operation. Preoperative examination had no obvious abnormality.

The treatment used High Intensity Focused Ultrasound equipment and transmission electron microscope. The experiment applied High Intensity Focused Ultrasound equipment, ultrasound transfection equipment, blood-gas analyzer and five kinds of complete blood cell analyzer.

Observation of dermohemia and edema by using different anesthetics

According to used anesthetics, patients were divided into three groups at random. Group A collected patients who conducted HIFU operation by using general anesthesia of A drug, Group B collected patients who conducted HIFU operation by using general anesthesia of B drug, and Group X collected patients who conducted HIFU operation by using general anesthesia of X drug. Record records were as follows: + for dermohemia, ++ for dermohemia accompanying with local mild swelling, and +++ for dermohemia accompanying with local serious swelling.

Experiment grouping of red blood cells in vitro, the number of red blood cells, and measurement of potassium concentration

In order to improve sensibility of red blood cells in the experiment, Na and K balanced solutions were used to dilute 10 times of whole blood specimens and reduce cell concentration of whole blood and coefficient of viscosity.

First of all, five kinds of complete blood cell analyzer and blood-gas analyzer were used to detect influences of N₂O on red blood cells. Transmission electron microscope was used to observe difference of red blood cells before and after inletting N₂O; the experiment detected influences of HIFU on red blood cells, confirmed experiment parameters, and conducted further experiment based on experiment parameters. The experiment of High Intensity Focused Ultrasound was divided into 4 groups, including control group (group C), nitrous oxide group (group N), pure HIFU group (group H), and HIFU plus nitrous oxide group (group H+N).

In the experiment of Low Intensity Focused Ultrasound, it detected influences of Low Intensity Focused Ultrasound on red blood cells, confirmed experiment parameters, and conducted further experiment based on experiment parameters. Low Intensity Focused Ultrasound was also divided into 4 groups, including control group (group C), nitrous oxide group (group N), pure Low Intensity Focused Ultrasound group (group L), and Low Intensity Focused Ultrasound plus nitrous oxide group (group N+L) to detect postprocessing results by using the same method.

Statistical analysis

The result of group A, B, X were analyzed by SPSS19.0 Ridit, the outcome of erythrocyte in vivo experiment showed by mean ± standard deviation (x±s), and were analyzed by SPSS19.0 t-test of two independent sample.

Results

Effect of different anesthetic drugs on the HIFU post-operation

As **Table 2**, the degree of skin swelling of group A was much higher than group B and group C. Analysis by SPSS19.0 Ridit turned out that the difference is statistically significant (P<0.05). Therefore, we considered that N₂O can enhance the effect of HIFU.

N₂O treatment increases the thickness of abdominal

In this study we observed the thickness of abdominal. The results indicated that the abdominal thickness before operation was sig-

Nitrous oxide enhances synergistic effects

Table 2. The effect of different anesthetic drugs on the HIFU post-operation

	A (N ₂ O + isoflurane + propofol + remifentanil) (n=32)	B (isoflurane + propofol + remifentanil) (n=33)	X (propofol + remifentanil) (n=32)	Chi square	P value
Mild hyperemia (+) (n, %)	0	8*	27 [#]	13.35	0.027
Moderate hyperemia and swelling (++) (n, %)	17	25*	3 [#]	10.06	0.042
Extreme hyperemia and swelling (+++) (n, %)	18	0*	0 [#]	18.43	0.015

Remarks: *Group A compared with group B, [#]P<0.05, and with group X P<0.05.

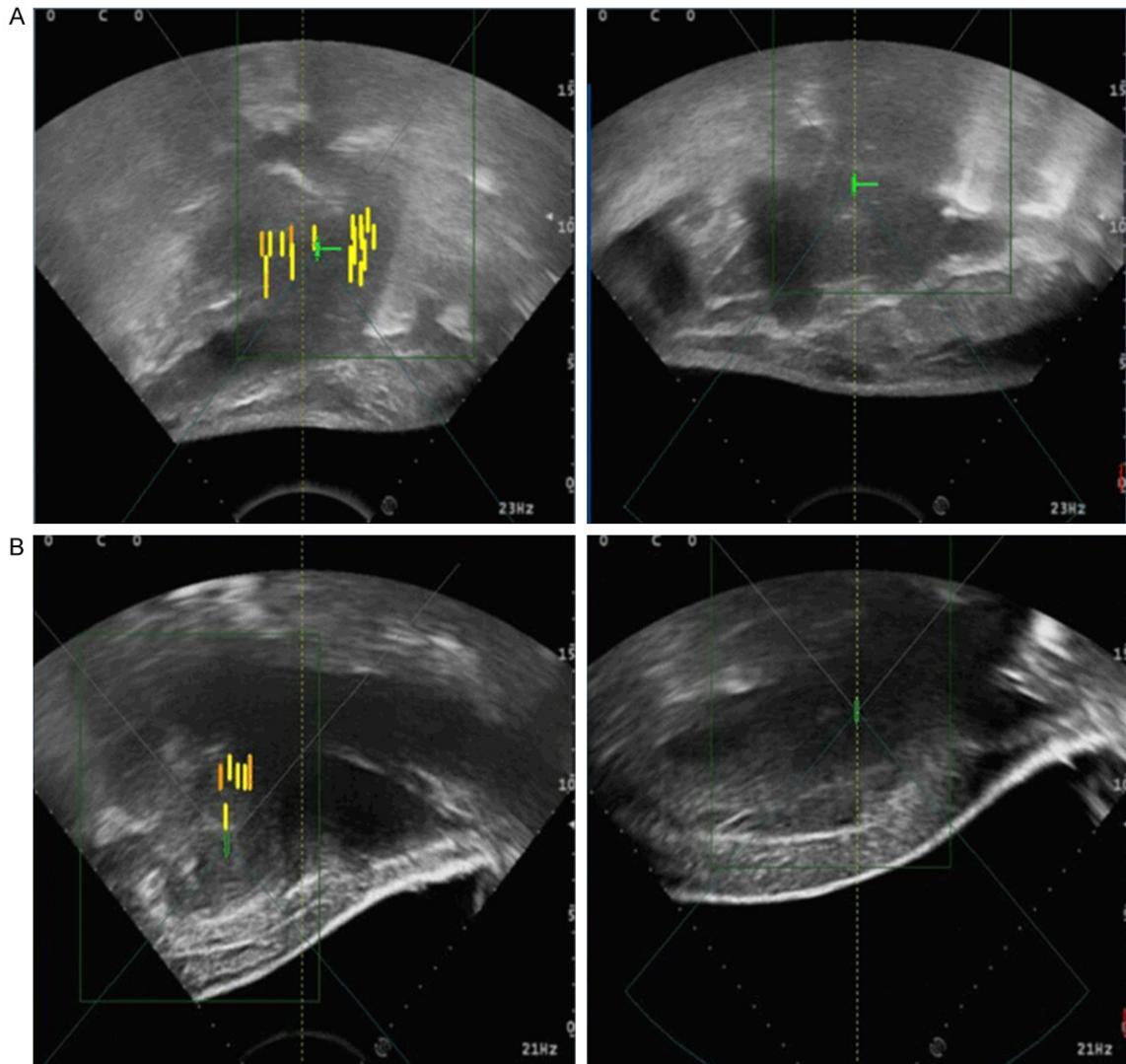


Figure 1. HIFU screenshot for the abdominal thickness pre-operation and post-operation. A. HIFU screen for group A. The left was pre-operation, and abdominal thickness was 2.5 cm. The right was one hour HIFU post-operation, and the abdominal thickness was 3.5 cm. B. HIFU screen for group B. The left was pre-operation, and abdominal thickness was 2.5 cm. The right was one hour post HIFU, and abdominal thickness was 2.5 cm.

nificantly increased compared to the abdominal thickness post operation in the group A (**Figure 1A**). However, there are no significant differences between the pre-operation and the post-operation in the group B (**Figure 1B**).

Effect of ventilation with N₂O in the erythrocyte suspension

There was no difference between before and after the ventilation with N₂O (the number of

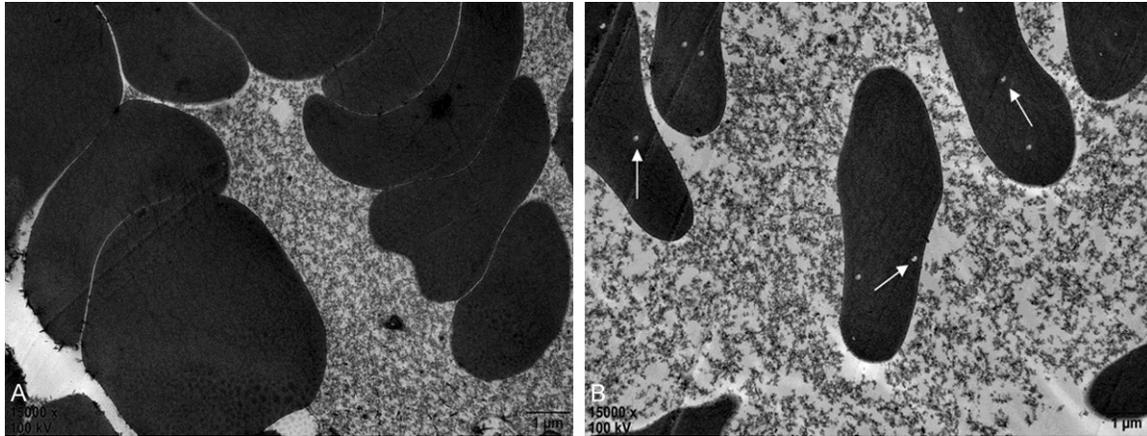


Figure 2. Observation for the micro-bubble in the erythrocyte using Transmission electron microscopy (TEM). A. Erythrocyte status before ventilation with N₂O. B. Erythrocyte status after ventilation with N₂O. The white arrow illustrates the micro-bubbles.

Table 3. The effect of N₂O combine with HIFU (150 W×5 s×2) on the erythrocyte and concentration of K⁺

	Number of erythrocyte (×10 ¹² /L)	Concentration of K ⁺ (mmol/L)
group C	0.365±0.007	5.45±0.092
group N	0.37±0	5.44±0.071
group H	0.218±0.083	6.464±0.198
group N+H	0.074±0.083*	7.002±0.177*

Group C: control group; group N: nitrous oxide group; group H: pure HIFU group; group H+N: HIFU plus nitrous oxide group. *P<0.05 represents the Number of erythrocyte or concentration of K⁺ in group N+H compared to group H.

Table 4. The effect of N₂O combine Low Intensity Ultrasound (150 W×5 s×2) on the erythrocyte and concentration of K⁺

	Number of erythrocyte (×10 ¹² /L)	Concentration of K ⁺ (mmol/L)
group C	0.355±0.006	4.055±0.055
group N	0.36±0.003	5.278±0.313
group L	0.345±0.008	4.445±0.471
group N+L	0.353±0.007*	4.465±0.415*

Group C: control group; group N: nitrous oxide group; group L: pure Low Intensity Focused Ultrasound group; group N+L: Low Intensity Focused Ultrasound plus nitrous oxide group. *P<0.05 represents the Number of erythrocyte or concentration of K⁺ in group N+L compared to group L.

erythrocyte (**Figure 2**). We conclude that N₂O will not damage cells. After the ventilation with N₂O, the number of micro-bubble in the erythro-

cyte was increased significantly in after ventilation with N₂O group (**Figure 2B**) compared to the before ventilation with N₂O group (**Figure 2A**) (P<0.05).

Effect of N₂O combines with HIFU on the erythrocyte

As shown of **Table 3**, there were significant differences between group H (HIFU group) and group N+H (N₂O combine with HIFU) on the number of erythrocyte (**Table 3**, F=0.01, P<0.05) and the concentration of K⁺ (**Table 3**, F=0.056, P<0.05).

Effect of N₂O combines with Low Intensity Ultrasound on the erythrocyte

In the experiment of N₂O combine with Low Intensity Ultrasound (remarks: parameter come from experiment of the effect on the Low intensity Ultrasound to erythrocyte), there were no differences between group L (Low Intensity Ultrasound) and group N+L (N₂O combine with Low intensity Ultrasound) on the number of erythrocyte (**Table 4**, P>0.05) and the concentration of K⁺ (**Table 4**, P>0.05). The results showed that N₂O combine with Low Intensity Ultrasound may not exist synergistic effect.

Discussion

In the therapy process of the HIFU, cavitation effect plays an important role [7]. Cavitation is the formation of vapour cavities in a liquid, such as small liquid-free zones, that are the

consequence of forces acting upon the liquid. Especially, under the effects of ultrasonic pressure wave, the liquid cavitation was formed. When the cavitation was damaged and broken, the energy was released rapidly, and following with the high temperature, high pressure, strong light and shock wave [8]. In the present study, the degree of skin swelling of group A was much higher than group B and group C. Analysis by SPSS19.0 Ridit turned out that the difference is statistically significant ($P < 0.05$). Therefore, we considered that N_2O can enhance the effect of HIFU.

In this study, we also observed the thickness of abdominal. The results indicated that the abdominal thickness before operation was significantly increased compared to the abdominal thickness post operation in group A. However, there are no significant differences between the pre-operation and the post-operation in the group B. Actually, the increased thickness of abdominal is a marker for the body injury, which could reflect the injury of HIFU on the body. This result confirmed that the N_2O could enhance the injury of abdominal. We speculated that when the N_2O enters into the body, the liquid is more easily to form the cavitation, which could also trigger the cavitation effects and aggravate the injury [9]. Furthermore, the N_2O could also strengthen the hot drama, enhancing the temperature surrounding the cells. When the temperature achieves beyond to the normal range, the cells would be broken and death [10]. Under the effects of N_2O in the cavitation process, the peroxy radical was obviously increased surrounding the cells, which could also increase the injury effects [11].

Flynn et al. divided the ultrasonic cavitation into two mainly types, including Steady cavitation and transient cavitation [12]. Actually, the steady cavitation and the transient cavitation could interconvert with each other and release large energy, when the strength of the ultrasonic pressure wave achieving to the threshold value. In the experiment of erythrocyte in vitro, there were significant differences between HIFU group and N_2O combine with HIFU group on the number of erythrocyte ($P < 0.05$) and the concentration of K^+ ($P < 0.05$). These results confirmed the effects of N_2O on the tissue injury. However, there were no significant differ-

ences between the two groups for the low Intensity Focused Ultrasound treatment (which may be caused by the steady cavitation in the Intensity Focused Ultrasound).

Hirokawa et al. [13] reported that there are some correlation between the HIFU treatment and the apoptosis of the cells. Saliev et al. [14] also proved that the HIFU treatment was associated with the cell apoptosis directly. Therefore, we speculated that the N_2O could strengthen the HIFU induced cell apoptosis, and aggravate the HIFU caused tissue injury.

In conclusion, application of nitrous oxide in HIFU operation aggravated injury degree of tissue. The combined utilization of nitrous oxide and HIFU played a synergistic effect on tissue injury and had the mechanism that N_2O increases micro bleb nucleon in tissue to reinforce ultrasonic cavitation effect and produce a synergistic effect.

Acknowledgements

This study was granted by the National Natural Science Fund of China (Grant No. 81172122). We thank Dr. Lan Hao and Dr. Pan Li in Chongqing Key Laboratory of Ultrasound Molecular Imaging.

Disclosure of conflict of interest

None.

Address correspondence to: Zedan Tian, Department of Anesthesiology, The Second Hospital Affiliated to Chongqing Medical University, Medical College Road 1, Yuzhong District, Chongqing 400016, P. R. China. E-mail: tianzedan@sina.com

References

- [1] Maleck WH, Piper SN, Koetter K. Joseph Priestley, nitrous oxide and oxygen. *Anaesthesist* 2005; 54: 395-396.
- [2] Smith WD. A history of nitrous oxide and oxygen anaesthesia. I. Joseph Priestley to Humphry Davy. *Br J Anaesth* 1965; 3: 790-798.
- [3] West JB. Humphry Davy, nitrous oxide, the Pneumatic Institution, and the Royal Institution. *Am J Physiol Lung Cell Mol Physiol* 2014; 307: L661-667.
- [4] Manouchehrian N, Bakhshaei MH. Nitrous oxide effect on relieving anxiety and pain in parturients under spinal anesthesia for caesarean section. *Anesth Pain Med* 2014; 4: 316662.

Nitrous oxide enhances synergistic effects

- [5] Jiang L, Hu B, Guo Q, Chen L. Sonographic and histological development of high-intensity focused ultrasound in rabbit muscle. *Exp Ther Med* 2013; 5: 33-38.
- [6] Shoji S, Nakano M, Fujikawa H, Endo K, Hashimoto A, Tomonaga T, Terachi T, Uchida T. Urethra-sparing high-intensity focused ultrasound for localized prostate cancer: Functional and oncological outcomes. *Int J Urol* 2015; 22: 1043-1049.
- [7] Cao H, Yin H, Qiao Y, Zhang S, Wan M. Sonochemiluminescence observation and acoustic detection of cavitation induced by pulsed HIFU at a tissue-fluid interface. *Ultrason Sonochem* 2013; 20: 1370-1375.
- [8] O'Neill BE, Vo H, Angstadt M, Li KP, Quinn T, Frenkel V. Pulsed high intensity focused ultrasound mediated nanoparticle delivery: Mechanisms and efficacy in murine muscle. *Ultrasound Med Biol* 2009; 35: 416-424.
- [9] Yu T, Wang G, Hu K, Ma P, Bai J, Wang Z. A microbubble agent improves the therapeutic efficiency of high intensity focused ultrasound: a rabbit kidney study. *Urol Res* 2004; 32: 14-19.
- [10] Umemura S, Kawabata K, Sasaki K. In vivo acceleration of ultrasonic tissue heating by microbubble agent. *IEEE Trans Ultrason Ferroelectr Freq Control* 2005; 52: 1690-1698.
- [11] Misik V, Riesz P. Peroxyl radical formation in aqueous solutions of N, N-dimethylformamide, N-methylformamide, and dimethylsulfoxide by ultrasound: implications for sonosensitized cell killing. *Free Radic Biol Med* 1996; 20: 129-138.
- [12] Flynn H. *Physics of Acoustic Cavitation in Liquids, Physical Acoustics*. W. P. Mason. New York: Academic Press; 1964. pp. 167.
- [13] Hirokawa N, Koito K, Okada F. High-intensity focused ultrasound induced apoptosis with caspase 3, 8, and 9/6 activation in rat hepatoma. *J Med Ultrason* 2009; 36: 177-185.
- [14] Saliev T, Feril LB, Nabi G, Melzer A. Targeted manipulation of apoptotic pathways by using High Intensity Focused Ultrasound in cancer treatment. *Cancer Lett* 2013; 338: 204-208.