様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　２ 年　 ７ 月　 １ 日現在

機関番号：３３９２０
研究種目：基盤研究(C)（一般）
研究期間：2017～2019
課題番号：１７Ｋ１１５９８

研究課題名（和文）ICU関連筋力低下における骨格筋前駆細胞の動態解析：間葉系幹細胞移植療法の応用

研究課題名（英文）The protective effect of mesenchymal stem cells on ICU-acquired muscle weakness

研究代表者
加納　秀記（Kano, Hideki）

愛知医科大学・医学部・教授

研究者番号：９０３４０２３１

交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：好中球をヒ素に暴露した際の、好中球機能を検討した。検討項目は、NETs（neutrophil extracellular traps)，活性酸素種 (ROS)産生能および貪食能である。 NETｓは、培養液中への、cell-free extracellular DNA (cf-DNA)，myeloperoxidase (MPO)-DNAおよび、 neutrophil elastase (NE)-DNAの放出量で確認した。その結果、いずれの項目もヒ素暴露により有意に減少しており、好中球機能障害の生じていることが明らかになった。

研究成果の学術的意義や社会的意義
ヒ素による、発がん性、血液障害、神経障害、易感染性が知られていたが、その病態生理は不明であった。また、好中球減少、マクロファージおよびリンパ球障害の生じることが奉公されていたが詳細な検討は行われていなかった。今回、in vitroの検討を行うことにより、好中球の様々な機能障害がヒ素により惹起されていることが明らかになった。本結果より、ヒ素による免疫毒性の一因として好中球機能異常の関連が推察された。

研究成果の概要（英文）：We evaluate the effects of NaAsO2 exposure on innate defense mechanisms regarding neutrophil extracellular traps (NETs), reactive oxygen species (ROS) production and phagocytosis in vitro. Polymorphonuclear leukocyte (PMN) were incubated with NaAsO2. NETs formation was quantified by measuring cell-free extracellular DNA (cf-DNA), myeloperoxidase (MPO)-DNA and neutrophil elastase (NE)-DNA. The medium level of cf-DNA, MPO-DNA and NE-DNA after stimulation with PMA were significantly reduced for PMN pretreated with arsenic compared with PMN without arsenic pretreatment. The PMN capacities of phagocytosis and ROS production were significantly reduced by arsenic pretreatment. We conclude that arsenic exposure causes neutrophils dysfunction. Our findings might provide a new insight in understanding the consequences of arsenic in inducing immunotoxicity and raising susceptibility to infectious diseases in human.

研究分野：救急医学

キーワード：NETｓ　貪食能　活性酸素種

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Arsenic, classified by the International Agency for Research on Cancer as a Group 1 carcinogen, is a major environmental toxicant and pollutant, and a global health concern. Its main sources are natural and anthropogenic. Arsenic compounds are frequently found in drinking water, foodstuffs, air, and soil, and elevated concentrations of arsenic in groundwater pose a huge threat to public health in some regions, such as Bangladesh, Taiwan, and Argentina. Humans and food-producing dairy animals inadvertently consume arsenic through contaminated drinking water and food. The Agency for Toxic Substances and Disease Registry ranked arsenic at the top of their Substance Priority List in 2019.

Arsenic intensifies non-infectious pathologies, including cancer, skin lesions, vascular diseases, diabetes mellitus, and neuropathy, but also damages hematological and immunological systems. Recent human and animal studies revealed that arsenic exposure causes neutropenia and damages lymphocyte and macrophage function, increasing susceptibility to recurrent opportunistic infections like tuberculosis as well as Influenza A and parasitic infections, thus acting as an immunosuppressive agent.

Brinkmann *et al*. first described a neutrophil-derived antimicrobial defense mechanism of innate immunity, termed neutrophil extracellular traps (NETs). Stimulation of NETs causes the extrusion of a meshwork of chromatin fibers expressing antimicrobial proteins like myeloperoxidase (MPO), neutrophil elastase (NE), and cathepsin G. NETs are essential in innate immunity and captures, immobilizes, and kills intruding gram-negative and gram-positive bacteria, fungi, and parasites, as well as invading microorganisms extracellularly. In a recent *in vitro* study, arsenic was shown to have potent granulotoxic effects in mammals and cause a marked decrease in phagocytosis by human and bovine neutrophils. But how arsenic exposure influences NET formation is yet to be investigated.

２．研究の目的

In the present study, we explored the possibility that NET formation by neutrophils would be altered under arsenic exposure. We further sought to examine phagocytosis, bactericidal activity, and generation of reactive oxygen species (ROS) to assess neutrophil function in the presence of arsenic.

３．研究の方法

Formation of NETs, production of ROS, and phagocytosis are evaluated. Polymorphonuclear neutrophils (PMNs) isolated from the peripheral blood of healthy human volunteers were incubated with or without 20 ng/ml $NaAsO_2$ for 12 h. Phorbol myristate acetate (PMA) was added to induce NET formation, which was quantified by measuring cell-free extracellular DNA (cf-DNA), MPO)-DNA and NE-DNA, and confirmed by immunofluorescence labeling and imaging. Extracellular killing of bacteria by NETs was evaluated by co-culturing *Escherichia coli* and PMNs in the presence of a phagocytic inhibitor.

４．研究成果

Levels of cf-DNA, MPO-DNA, and NE-DNA in the culture medium after PMA stimulation were significantly lower in PMNs pre-exposed to arsenic than those not exposed to arsenic. Immunofluorescence staining to examine released DNA, NE, and MPO and extracellular killing of bacteria by NETs revealed similar results. Phagocytosis and ROS production by PMNs were also significantly reduced by arsenic pre-exposure. We conclude that acute low-dose arsenic exposure *in vitro* causes neutrophil dysfunction in terms of NET formation, phagocytosis, and ROS production. Our findings provide new insights into the mechanisms of arsenic immunotoxicity and how it increases susceptibility to infectious diseases in humans. This is the first study to demonstrate how acute low-dose arsenic exposure *in vitro* impairs human PMN function, affecting NET formation, phagocytosis, and ROS production. PMA-stimulated release of MPO-DNA, NE-DNA, and cf-DNA into the cell culture medium was significantly lower in arsenic-pre-exposed PMNs than in PMNs not exposed to arsenic, indicating that NET formation was impaired. NET-related bacterial killing, which was evaluated in the presence of a phagocytosis inhibitor, was also significantly reduced in arsenic-pretreated PMNs. Immunofluorescence staining of released DNA, NE, and MPO confirmed these results. Phagocytosis and PMA-stimulated ROS production, which are other important functions of PMNs, were also impaired by arsenic.

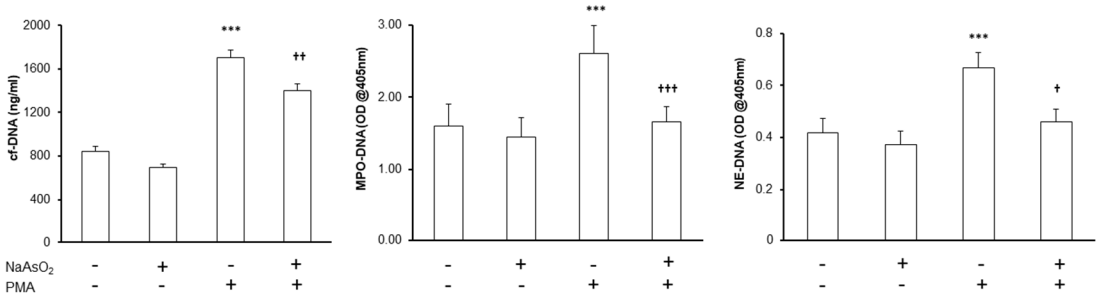
The arsenic-induced impairments we observed in PMN functions, including phagocytosis and ROS production, are consistent with previous data. Taheri *et al*. showed that exposing PMNs to arsenic *in vitro* significantly decreased both phagocytosis and PMA-induced hydrogen peroxide production. They concluded that arsenic possesses granulotoxic properties and may impair innate immunity. In zebrafish, PMA-induced ROS production assessed by oxidation of dihydrodichlorofluorescein diacetate to dichlorofluorescein was also decreased upon arsenic exposure.

Humans and mice exposed to arsenic show elevated incidence and severity of infections, respiratory problems, and fungal and parasitic infections. In mice, chronic arsenic exposure resulted in a severely compromised response to influenza A infection accompanied by decreased cytokine production and increased mortality. In Bangladesh, prenatal arsenic exposure from drinking water was found to increase the risk of lower respiratory tract infection during infancy. Other studies have shown that *ex vivo* peripheral blood mononuclear cell proliferation and IL-2 secretion were reduced in arsenic-exposed individuals. The

impairments in PMN function observed in the present study would explain, at least in part, the enhanced risk of immune dysregulation and infection in arsenic-exposed individuals.

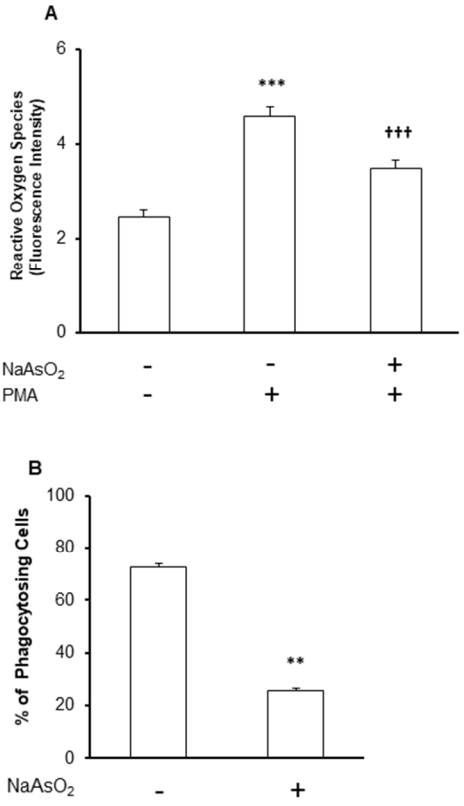
cf-DNA, MPO-DNA, and NE-DNA levels after exposure of PMNs to arsenic

Data are presented as the mean ± SEM (n = 10). ***p < 0.001 vs. $NaAsO_2$(−)/PMA(−); †p < 0.05, ††p < 0.01 and †††p < 0.001 vs. $NaAsO_2$(−)/PMA(+).



ROS production and phagocytic capacity in PMNs after arsenic exposure.

(A) ROS production after PMA stimulation of isolated PMNs pretreated without or with arsenic. Data are presented as the mean ± SEM (n=10). ***p < 0.001 vs. $NaAsO_2$(−)/PMA(−); †††p < 0.001 vs. $NaAsO_2$(−)/PMA(+). (B) Summarized FACS results of phagocytic capacity in PMNs after arsenic exposure. Data are presented as the mean ± SEM (n = 5). **p < 0.01 vs. $NaAsO_2$(−).



5．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　2件）

1．発表者名 丸地祐樹、後長孝佳、竹中信義、森久剛、Huq MA、津田 雅庸、武山 直志
2．発表標題 血中myeloperoxidase-conjugated DNA測定による敗血症性ショック予後予測の検討
3．学会等名 第33回日本shock学会総会
4．発表年 2018年

1．発表者名 Takeyama N, Gocho T, Maruchi Y, Takenaka N, Mori H, Islam Md M, Huq MA
2．発表標題 Removal of circulating NETs-related components with an immobilized polymyxin B filter
3．学会等名 39th International symposium on Intensive Care and Emergency medicine（国際学会）
4．発表年 2019年

1．発表者名 Md. Monirul Islama and Naoshi Takeyamaa
2．発表標題 Low-dose acute inorganic arsenic exposure suppresses human neutrophil function in vitro
3．学会等名 33rd ESICM（国際学会）
4．発表年 2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究分担者	武山　直志 (Takeyama Naoshi) (00155053)	愛知医科大学・医学部・教授 (33920)	

６．研究組織（つづき）

	氏名 （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究分担者	竹中　信義 (Takenaka Nobuyoshi) (60770534)	愛知医科大学・医学部・助教 (33920)	
研究分担者	富野　敦稔 (Tomino Atunori) (70440980)	愛知医科大学・医学部・講師 (33920)	