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研究課題名（和文）ドラッグ・リポジショニングを応用した急性呼吸窮迫症候群に対する薬物療法の開発

研究課題名（英文）Development of pharmacological therapy of ARDS by drug repositioning

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研究成果の概要（和文）：集中治療医学の進歩にも関わらず、急性臓器不全に対する治療法は身体の恒常性をできるだけ正常な状態に保つように努めながら、臓器の回復も待つ保存的療法であり、臓器の回復そのものを促進する薬物療法は未だ確立されていない。一方、最近、既存薬の本来の薬効とは異なる効果に着目して治療に応用する－Drug Repositioning－という方法が着目されいている。本研究では、金属である塩化スズがストレス蛋白誘導効果を有することに着目してラット横紋筋融解症性急性腎傷害に対する塩化スズの投与がストレス蛋白誘導を介して急性腎傷害を改善すること明らかにした。

研究成果の学術的意義や社会的意義
近年、気候変動の影響で大規模災害が多発している。災害に伴う建物の倒壊により下敷きになった被災者は救助することができても途絶した血流の再開によって筋肉が破壊され、そこから遊離した毒物によって腎臓が障害され致死的状態（横紋筋融解症性急性腎傷害）に至ることがある。しかし、この病態に対して決め手となる薬物は開発されていない。一方、新薬を開発するプロセスは多大であることから、新規の薬物を開発することなくこれまで存在している薬物を本来とは異なる病気に応用するドラッグ－リポジショニング(DR)という手法が最近注目されている。本研究はDRを応用した重症病態に対する薬物開発の端緒を開くものである。

研究成果の概要（英文）：Despite recent progress in critical care, there have been no pharmacological modalities against acute organ injury although supportive therapeutic modalities have been developed. Drug repositioning, which is a process of identifying new therapeutic use(s) for old/existing/available drugs, has become a popular strategy in recent years. It is an effective strategy in discovering or developing drug molecules with new pharmacological/therapeutic indications. Although tin chloride is not a drug but a heavy metal toxic to humans it is known that tin chloride induces heme oxygenase-1; a stress protein, in a kidney specific manner. We administered tin chloride to a rat model of rhabdomyolysis-induced acute kidney injury produced by glycerol injection and examined its effect on the kidney injury. We found that tin chloride treatment significantly ameliorates the kidney injury by virtue of the induction of heme oxygenase-1 without any adverse effect in rats.

研究分野：麻酔蘇生学

キーワード：急性臓器障害　急性腎傷害　横紋筋融解症　ヘムオキシゲナーゼ　塩化スズ　遊離ヘム　Bach1　ALAS1

１．研究開始当初の背景

Rhabdomyolysis and associated acute kidney injury (RM-AKI) is a severe disorder. However, no definitive treatment for this disease has been established. Using a rat model of RM-AKI induced by glycerol (Gly) injection, we previously reported that free heme, a pro-oxidant released by myoglobin derived from rhabdomyolysis has an deleterious role in pathogenesis of this disease by evaluating dynamic changes in the expression of next three substances; Heme oxygenase-1 (HO-1), a rate-limiting enzyme of heme catabolism, delta-aminolevulinate synthase (ALAS1, a heme biosynthetic enzyme), and BTB and CNC homology 1 (Bach1), an inhibitory transcription factor of HO-1 (1). It has been reported that administration of small amount of hemoglobin induces HO-1 and have a protective effect on RM-AKI (2). However, the administration of hemoglobin may cause the increase in free heme, a harmful substance to human body. On the other hand, we have reported that tin chloride ($SnCl_2$) is a kidney specific HO-1 inducer and has a protective effect on a rat model of AKI induced by ischemia-reperfusion (3). Furthermore, $SnCl_2$ has been reported to induce HO-1 in free heme independent manner (4).

２．研究の目的

The aim of the present study is to examine whether $SnCl_2$ has a protective effect on a rat model of RM-AKI mediated through the reduction in free heme concentration in the kidney.

３．研究の方法

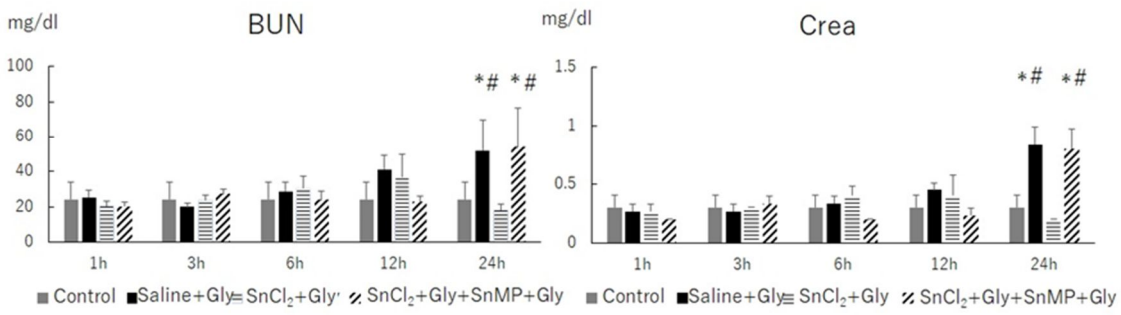
This study was approved by the animal use and care committee of Okayama University Medical School. Male Sprague-Dawley rats (7 weeks old) were divided into 4 groups (n = 4 in each group); Control group (untreated), $SnCl_2$+Gly group, Saline+Gly group, $SnCl_2$+SnMP (Tin mesoporphyrin; a specific inhibitor of HO) +Gly group. $SnCl_2$+Gly group were injected with $SnCl_2$ (100 mg/100 g) subcutaneously and after 24 h injected with 50% Gly (10 ml/kg) into bilateral hind limbs. Saline+Gly group were injected with the same amount of saline followed by the Gly injection. $SnCl_2$+SnMP+Gly group were additionally injected with SnMP (1 μmol/kg) 1 h before Gly injection to $SnCl_2$+Gly rats. Blood and kidneys were collected after Gly injection at each timepoint (1,3,6,12,24 h). Serum BUN and Creatinine (Crea) level were measured as indicators of renal function. Histological analysis was performed by hematoxylin and eosin (HE) staining 24 h after Gly injection with the scoring of tubular injury. Gene expression of HO-1 and ALAS1 was evaluated by northern blot analysis, and protein level of HO-1 in cytosol and Bach1 in nucleus was evaluated by western blot analysis.

４．研究成果

After 24 h of Gly injection serum, BUN and Crea levels of Saline+Gly group were significantly increased compared with Control group. In contrast, those of $SnCl_2$+Gly group were significantly lower than Saline+Gly group. However, additional treatment of SnMP abolished the beneficial effect of $SnCl_2$. (Fig. 1). Histological damage observed in Saline+Gly group was significantly improved by $SnCl_2$ treatment whereas HO inhibition by SnMP abrogated the improvement by $SnCl_2$ treatment. Those observation was supported by the results of tubular injury score. (Fig. 2). HO-1 mRNA level at 6 h after the Gly injection was significantly higher in $SnCl_2$+Gly group than Saline+Gly group (Fig. 3). Renal ALAS1 mRNA level and renal nuclear Bach1 protein levels of Saline+Gly group were significantly lower than control group at 3 h after Gly injection. On the other hand $SnCl_2$ treatment reversed these levels almost to control levels. (Figs. 4 and 5).

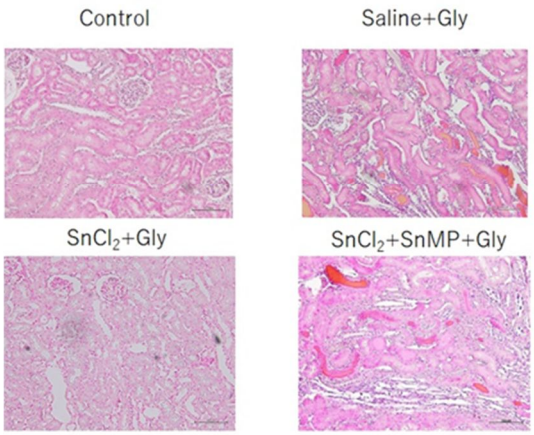
In conclusion, these findings suggest that the administration of $SnCl_2$ have a protective effect on RM-AKI by induction of HO-1. The increase in intracellular free heme level is known to down-regulate ALAS1 and translocate Bach1 from nucleus to cytosol to activate HO-1 transcription. $SnCl_2$ treatment dampened changes in ALAS1 and Bach1 induced by Gly treatment, suggesting the decrease in intracellular free heme levels. Thus, the reduction of free heme can be implicated in the protective effect of $SnCl_2$ treatment.

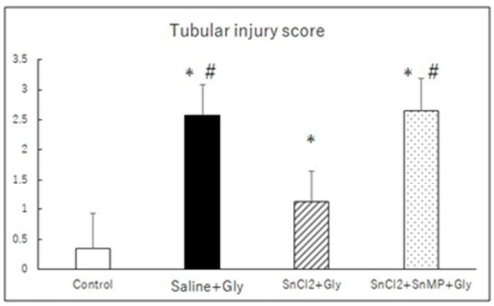
Figure. 1 Serum BUN and Creatinine levels



Statistical analysis was performed by analysis of variance followed by Tukey-Kramer multiple comparisons Method.
*Significantly different from untreated control rats($P<0.005$).
#Significantly different from rats $SnCl_2$ treated and after 24h glycerol treatment(<0.005)

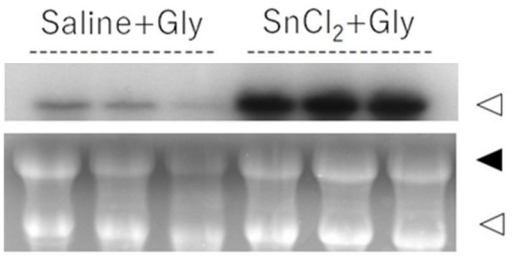
Figure. 2 Histological analysis by HE staining

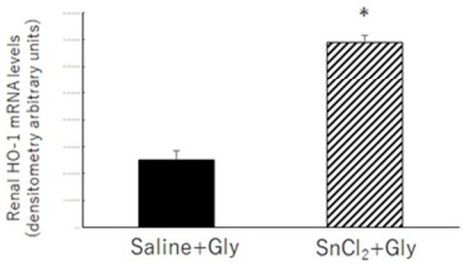




Tubular injury score was graded by estimating the percentage of injured tubules as follows: 0, areas of injured tubules < 5%; areas of injured tubules 5%-25%; 2, areas of injured tubules 26%-50%; 3, areas of injured tubules 51%-75%;4, areas of injured tubules > 75%.
Statistical analysis was performed by analysis of variance followed by Tukey-Kramer multiple comparisons Method.
*Significantly different from control($p<0.005$)
#Significantly different from $SnCl_2$ group($p<0.005$)

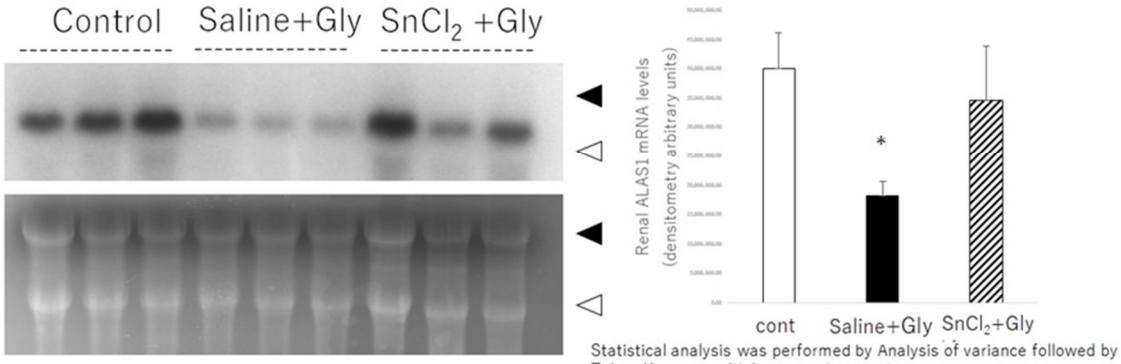
Figure. 3 HO-1 mRNA level 6 h after Gly treatment





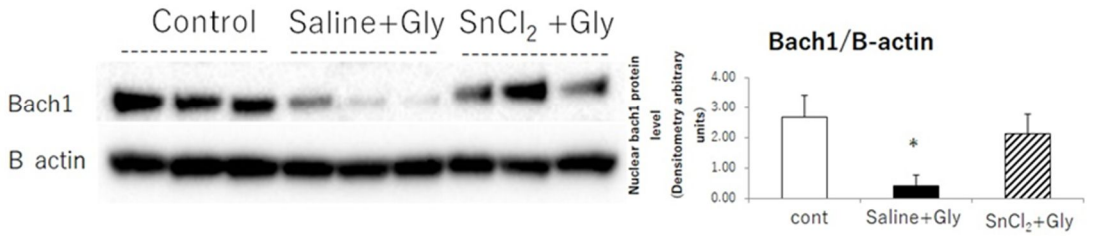
Statistical analysis was performed by unpaired student's t-test
*significantly different from saline group($p<0.05$)

Figure 4. ALAS1 mRNA level



Statistical analysis was performed by Analysis of variance followed by Tukey-Kramer multiple comparison test.
* Significantly different from control and gly+$SnCl_2$ groups ($p<0.05$)

Figure. 5 Renal Bach1 protein level in nucleus



Statistical analysis was performed by Analysis of variance followed by Tukey-Kramer multiple comparison test.
* Significantly different from control and $SnCl_2$ groups($p<0.05$)

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4. Biochem J.1993;290:819-825

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　2件）

１．著者名 Ohtani Shinkichi、Shimizu Hiroko、Yamaoka Masakazu、Takahashi Toru、Omori Emiko、Morimatsu Hiroshi	４．巻 17
２．論文標題 Protective effect of tin chloride on rhabdomyolysis-induced acute kidney injury in rats	５．発行年 2022年
３．雑誌名 PLOS ONE	６．最初と最後の頁 e0265512
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pone.0265512	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Tanioka Nohito、Shimizu Hiroko、Omori Emiko、Takahashi Toru、Yamaoka Masakazu、Morimatsu Hiroshi	４．巻 75
２．論文標題 Role of the Transcription Factor BTB and CNC Homology 1 in a Rat Model of Acute Liver Injury Induced by Experimental Endotoxemia	５．発行年 2021年
３．雑誌名 Acta Medica Okayama	６．最初と最後の頁 363～372
掲載論文のDOI（デジタルオブジェクト識別子） 10.18926/AMO/62232	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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７．科研費を使用して開催した国際研究集会

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８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関