The Plasma Concentration of MUC5B Is Associated with Clinical Outcomes in Paraquat-poisoned Patients

To the Editor:

Paraquat (*N,N'*-dimethyl-4,4'-bipyridinium dichloride), a nonselective contact herbicide, is a common cause of poisoning in developing countries. Until the recent ban, paraquat was the most common cause of fatal self-poisoning in Sri Lanka. Ingestion leads to the development of multiorgan failure, severe pulmonary fibrosis, and death (1, 2). Paraquat-induced pulmonary fibrosis partly results from the direct damage caused by oxygen free radicals as well as indirect injury caused by inflammatory cells and fibroblasts. Mortality rates remain greater than 50% in self-poisoned patients because of the lack of an efficient antidote and limited response to antiinflammatory or antioxidant treatment (1, 3).

The *MUC5B* (mucin 5B, oligomeric mucus/gel-forming) gene encodes respiratory tract mucin glycoproteins that play a role in mucociliary clearance and host defense (4). We have previously shown that a common, gain-of-function *MUC5B* promoter polymorphism (rs35705950) is the strongest risk factor for the development of idiopathic pulmonary fibrosis (IPF) (5, 6). Moreover, among patients with IPF, the gain-of-function *MUC5B* promoter variant was significantly associated with improved survival (7). At this time, the best predictor of survival after self-poisoning by paraquat is time from ingestion and plasma paraquat concentration, termed the severity index of paraquat poisoning (SIPP score) (8). The objective of the present study is to determine whether the concentration of plasma MUC5B is associated with mortality in patients poisoned by paraquat.

We obtained plasma samples from a previously described cohort of self-poisoned patients with paraquat toxicity in Sri Lanka (3, 8, 9). Briefly, patients ingesting paraquat were enrolled at 10 different Sri Lankan hospitals

Supported in part by National Institute of General Medical Sciences grant 1K23GM110516-01 and NIH/National Center for Advancing Translational Sciences Colorado Clinical Translational Science Institute grant UL1 TR000154 (A.A.M.), National Natural Science Foundation of China Fund grant 81671899 (H.S.), National Health and Medical Research Council of Australia Program grant 1055176 (N.A.B.), and NIH grants R01 HL097163, P01 HL092870, R21/R33 HL120770, UH2/3-HL123442, and 1U01BX001534 (D.A.S.).

Author Contributions: A.A.M. conceived the study, brought together the collaboration, and drafted the manuscript; H.S. developed the MUC5B assay, performed the protein quantification, and helped with manuscript drafting and finalization; A.M.R.-O. performed the statistical analysis; F.M. and I.G. enrolled patients in the paraquat poisoning cohort and provided plasma samples for analysis; N.A.B. conceived the paraquat poisoning studies and directed the research staff for this effort; C.M.E. provided methodological guidance for development of the MUC5B protein assay; I.V.Y. oversaw the assay development and contributed to troubleshooting the assay; D.A.S. is the principal investigator who identified the role of MUC5B in pulmonary fibrosis, assisted in the analysis and writing of the manuscript, and provides funding for the laboratory; all authors have reviewed, contributed to writing the manuscript, and approved the final product.

Originally Published in Press as DOI: 10.1164/rccm.201705-0866LE on July 18, 2017

between April 2005 and January 2008. Blood was obtained from patients on admission or during the patient's first hospital day, and plasma paraquat levels were measured.

MUC5B from human saliva was used to generate a standard curve. Dot blot analysis on the plasma sample was performed using S&S Minifold I dot blot filtration manifold (Schleicher and Schuell). Samples were diluted 1:100 and run in duplicate along with the standard curve, also run in duplicate. The protein samples were allowed to bind to polyvinylidene difluoride membrane, the membrane was blocked at room temperature in 5% milk in phosphate-buffered saline with Tween for 1 hour then incubated overnight at 4° with the MUC5B primary antibody (Santa Cruz Biotechnology) diluted 1:5000 with 2.5% milk. Secondary goat anti-rabbit IgG biotin conjugate in 2.5% milk (1:2000 dilution) was applied for 1 hour at room temperature followed by incubation with strep-horseradish peroxidase (Life Technologies). Blots were developed using the horseradish fluoroluminescence detection protocol, using SuperSignal Dura Extended Duration Substrate (Pierce).

We used a multivariate Cox proportional hazards model for survival analysis. We used the baseline SIPP score, which is the product of the time since ingestion and the serum paraquat concentration at admission (8), as the

Table 1. Demographics and Case Characteristics of Patients Self-poisoned by Paraquat

Age, yr	
<20	14 (15.7%)
20s	40 (44.9%)
30s	23 (25.8%)
40s	4 (4.5%)
50s	5 (5.6%)
60+	3 (3.4%)
Sex	
Female	22 (24.7%)
Male	67 (75.3%)
In-hospital death	
No	42 (47.2%)
Yes	47 (52.8%)
Death within 3 mo	
No	28 (31.5%)
Yes	61 (68.5%)
Sampling time from ingestion, h	
Mean (SD)	9.5 (7.35)
Median	7.1
Range	0.9 to 38.5
N	89
Baseline severity index of paraquat poisoning	
Mean (SD)	64.4 (235.80)
Median	9.3
Range	0.1 to 1,936
N	89
Geometric mean (95% confidence interval)	7.49 (4.68 to 11.99)
MUC5B protein concentration in µg/ml	
Mean (SD)	−0.0866 (0.0332)
Median	0.076
Range	−0.021 to −0.202
N	89

Definition of abbreviation: MUC5B = mucin 5B.

Figure 1. Paraquat self-poisoning survival by MUC5B (mucin 5B) and log of baseline severity index of paraquat poisoning (SIPP). The median values of MUC5B (-0.0759) and log of baseline SIPP (2.22) are used to distinguish the four groups.

independent paraquat variable. The time until death or final follow-up was modeled as a function of the natural log of the baseline SIPP score, the MUC5B concentration, and the interaction of the log of the baseline SIPP and MUC5B. Sex and age were found to be nonsignificant and are therefore not included in the model. We calculated hazard ratios for the risk for death.

Demographics, baseline characteristics, MUC5B measurements, and SIPP scores of the 89 cases are summarized in Table 1. Figure 1 illustrates the survival probability based on MUC5B quantity and the log of baseline SIPP. The curve for patients with a log of baseline SIPP measurement below the median stabilizes around 63% for patients with a higher-than-median MUC5B plasma concentration and around 56% for patients with a lower-than-median MUC5B plasma concentration. Patients with a higher baseline SIPP have significantly worse outcomes, with survival probabilities leveling off around 5% for those with a lower-than-median MUC5B. All 26 patients who had a higher-than-median baseline SIPP measurement and a higher-than-median MUC5B died within the first 3 months.

The Cox proportional hazards model demonstrates a hazard ratio of 0.78 (95% confidence interval [CI], 0.64–0.95) for MUC5B, suggesting a lower risk for death with higher MUC5B, and 0.93 (95% CI, 0.56–1.56) for the log-baseline SIPP score. The interaction of the plasma concentration MUC5B and log-baseline SIPP has a hazard ratio of 1.11 (95% CI, 1.04–1.19), suggesting that among patients with a low baseline SIPP, those with a higher quantity of MUC5B have higher chances of survival than those with a lower MUC5B. Among the relatively few patients with a high baseline SIPP, those with a higher concentration of MUC5B have lower chances of survival. Overall, high MUC5B concentrations improve prognosis except in those with very high SIPP scores. This suggests that higher paraquat concentrations may simply overwhelm the

protective effects of MUC5B. In the future, we will seek to confirm these observations in animal models through overexpression and knock-out approaches.

Our findings demonstrate that MUC5B concentrations in the peripheral blood are associated with mortality in patients self-poisoned by paraquat. The amount of paraquat ingested and the time to presentation, as reflected by the SIPP score, remains the most predictive factor for subsequent mortality (8). However, the interaction of SIPP baseline score with MUC5B concentration suggests MUC5B may provide some protection from paraquat-induced mortality. These data paired with the prior knowledge that the MUC5B promoter variant rs35705950 is associated with improved survival in IPF (6) suggest that the MUC5B protein may be protective in oxidative injury leading to pulmonary fibrosis.

The persistently high mortality rate in paraquat self-poisoning (3, 10) highlights the need for improved treatment approaches. Pulmonary mucins may be a future therapeutic target for these patients. In addition, MUC5B may be a prognostic tool in poisoned patients with known timing and paraquat concentrations.

Lack of pulmonary imaging in this study limits our ability to associate the MUC5B concentrations with acute pulmonary toxicity, although mortality is arguably the most clinically important variable. We were unable to genotype these subjects because the purified plasma specimens contained very few peripheral blood mononuclear cells, which increased the difficulty of DNA extraction. Finally, lack of long-term follow up for pulmonary toxicity in the survivors minimizes our ability to comment on the development of paraquat-associated pulmonary fibrosis and the role of MUC5B in this pathology. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Downregulation of MicroRNA-126 Augments DNA Damage Response in Cigarette Smokers and Patients with Chronic Obstructive Pulmonary Disease

To the Editor:

Increased DNA damage response (DDR) contributes to the pathophysiology of aging disorders, including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and cancer (1, 2). DNA damage resulting from cigarette smoke-induced oxidative stress activates the DDR signaling pathway, controlled mainly by the ATM (ataxia-telangiectasia mutated) protein kinase. Accumulation of DNA damage promotes cellular senescence and disrupts tissue homeostasis (2). Using circulating endothelial precursors called blood outgrowth endothelial cells (BOEC) or endothelial colony-forming cells, we have previously demonstrated increased DDR, endothelial senescence, and dysfunction in smokers and patients with COPD (3), supporting the concept of accelerated aging of the endothelium as contributing to CVD in these groups.

MicroRNAs are important regulators of almost every cellular process, including DNA repair. MicroRNA-126 (miR-126; also known as miR-126-3p), a microRNA enriched in endothelial cells, plays a key role in angiogenesis and vascular homeostasis (4). miR-126 is reduced in patients susceptible to developing or with CVD (5–7) and in several types of cancer, including lung cancer (8). The link between miR-126 and DDR signaling in COPD is currently unknown. Therefore, we investigated whether miR-126 is reduced by cigarette smoke *in vivo* and in endothelial and lung epithelial cells from patients with COPD, as well as its link to DDR activation.

Methods

To investigate DDR resulting from cigarette smoke *in vivo*, male C57BL/6 mice were challenged for 28 days with cigarette smoke or ambient air, as previously described (9). BOEC were isolated from human blood samples, and human primary bronchial epithelial cells were isolated from lung tissue as described (3, 10). Informed consent was obtained from all individuals (Table 1). Data have been derived from subjects and animals used in previous reports (3, 9, 10). Human umbilical vein endothelial cells were transfected with miRCURY

This work was supported by a Wellcome Trust Programme Grant (093080/Z/10/Z). K.E.P. is financially supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. A.Z. and R.D.S. were financially supported by the British Heart Foundation (BHF). M.M. is a BHF Chair Holder, and his research is supported by the BHF, NIHR Biomedical Research Centre based at Guy's and St. Thomas' NHS Foundation Trust and King's College London, and Fondation Leducq Transatlantic Network of Excellence. A.M.R.'s research is supported by a BHF programme grant and the BHF Centre for Vascular Regeneration (Rm/30158).

Author Contributions: Conception and design: K.E.P., A.M.R., and P.J.B.; data analysis and interpretation: K.E.P., A.Z., J.R.B., M.A.B., R.D.S., M.G.B., L.E.D., M.M., A.M.R., and P.J.B.; experimental performance: K.E.P., A.Z., and J.R.B.; and writing: K.E.P., A.M.R., and P.J.B.

Originally Published in Press as DOI: 10.1164/rccm.201706-1304LE on July 28, 2017