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In Vitro Evaluation of the Interaction Between Activated Charcoal and *N*-Acetylcysteine after Acetaminophen Adsorption

Yoshinori Tomoda,* and Mariko Fukumoto

Laboratory of Clinical Toxicology, Research and Education Center for Clinical Pharmacy, Kitasato University School of Pharmacy, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
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Gastrointestinal decontamination by activated charcoal (AC) is the most important treatment for acetaminophen (APAP) overdose. Because AC adsorbs a wide variety of toxins, it may also adsorb the oral antidote, *N*-acetylcysteine (NAC). NAC is a specific antidote for APAP overdose and administered as a 72-h oral regimen. We evaluated AC adsorption of NAC after APAP adsorption *in vitro*. Different concentrations of NAC solution diluted with simulated gastric fluids (SGF) and simulated intestinal fluids (SIF) were added to AC and incubated at 37°C for 1 h. The AC was then removed by filtration, and the NAC concentration was determined. This revealed that NAC was not only adsorbed onto the AC but also converted to *N,N'*-diacetyl-L-cystine (DAC), which is oxidized NAC. We then calculated the maximum adsorption capacity per gram of AC (*Qm*). The apparent *Qm* based on the amount of decreased NAC in the SGF was 400 mg/g, and that in the SIF was 714 mg/g. The actual *Qm* based on only the amount of adsorption in the SGF was 294 mg/g, and that in the SIF was 59 mg/g. We also determined whether or not AC could adsorb the loading and maintenance doses of NAC after APAP adsorption. The residual rate in the SGF was 2.1%, and that in the SIF was 0.3%. The rate of conversion to DAC was higher in the SIF than that in the SGF. By both the actions of adsorption and oxidation, AC may reduce the effect of loading and maintenance doses of NAC.

Key words activated charcoal, acetaminophen overdose, acetylcysteine, oral antidote, adsorption

INTRODUCTION

Activated charcoal (AC) is an adsorbent that can adsorb a variety of drugs, poisons, and chemicals. It is widely used for accidental or intentional ingestion of these substances as a first-line treatment. Many studies have demonstrated the ability of AC adsorption *in vitro* and reducing bioavailability *in vivo*. If AC is given within 60 min after ingestion, its efficacy is significantly increased.[1)]

It is well known that AC is an effective agent for acetaminophen (APAP) overdose.[2–5)] APAP is commonly used as an antipyretic and an analgesic. It is also a common agent in drug overdoses due to its availability in over-the-counter preparations. Because APAP overdose causes liver injury and often death in severe cases, it is important that AC is administered soon after APAP ingestion as a first treatment. It is also important in *N*-acetylcysteine (NAC) therapy. NAC is used as a specific antidote for APAP overdose to help prevent liver injury. It is a precursor of glutathione (GSH) that detoxifies *N*-acetyl-*p*-benzoquinone imine (NAPQI), a highly reactive intermediate from APAP.[6,7)] It is approved for a 72-h oral regimen, with a loading dose of 140 mg/kg, followed by 70 mg/kg every 4 h, for an additional 17 doses.[8)] The earlier the administration of NAC, the greater the likelihood of preventing hepatotoxicity. It should be started as soon as possible but is effective within 8–24 h after APAP ingestion.[9)]

Rumack and Peterson reported the 72-h oral NAC regimen approved by the FDA.[8)] In their study, the protocol did not include AC treatment because AC would decrease the absorption of NAC. Although the evidence is not clear, it is recommended in the package insert that NAC should be administered at least 1 h after AC has been given to avoid decreasing the absorption of NAC.[10)] However, the transit time of AC is several hours, even though a cathartic is given with the AC to enhance the elimination of the charcoal-poisoning complex.[11,12)] If AC stays in the stomach and intestines, the NAC would likely be adsorbed onto the AC. The objective of this study was to evaluate the effect of AC on a 72-h oral regimen of NAC using *in vitro* methods.

MATERIALS AND METHODS

Medicinal carbon powder, NAC, NAC oral solution 17.6%, and *N,N'*-diacetyl-L-cystine (DAC) were obtained from Nichi-Iko Pharmaceutical Co., Ltd. (Tokyo, Japan), Wako Pure Chemical Corporation (Osaka, Japan), Ayumi Pharmaceutical Corporation (Tokyo, Japan), and Bachem AG (Bubendorf, Switzerland), respectively. All other chemicals or solvents were of analytical grades. The Plus shaker EP-1 (Taitec, Saitama, Japan), the Thermo minder SM-05N (Taitec), and the pH meter F-52 (Horiba, Kyoto, Japan) were used. The filters used qualitative filter paper and cellulose acetate membrane filters (Advantec, Tokyo, Japan). Purified water was prepared by Smart2Pure (Thermo Fisher Scientific K.K., Tokyo, Japan).

*To whom correspondence should be addressed. e-mail: tomoda@kitasato-u.ac.jp

Simulated gastric fluid (SGF) at pH 1.2 and simulated intestinal fluid (SIF) at pH 6.8 were prepared according to the JP XVII. The APAP solution was prepared by dissolving 10 g of APAP in 1 L of SGF and SIF, respectively. Stock solutions of NAC (0.25, 0.5, 1–10 mg/mL, and 5%) were prepared from an NAC oral solution by diluting it with SGF and SIF, respectively. APAP, NAC, and DAC standard solutions for calibration curves were prepared by dissolving their respective reagents in water (1, 1, and 0.5 mg/mL, respectively) and then diluted with 10, 25, 50 μg/mL of APAP and 5, 10, 25 μg/mL of each NAC and DAC, respectively.

Calculations of the adsorption of NAC onto AC in the SGF and SIF were carried out *in vitro*. After placing 125-mg amounts of AC powder into bottles, 20 mL of each stock solution of NAC (0.25–10 mg/mL) was added to the bottles. We then incubated the suspensions in a shaker at 37°C water bath for 1 h and then let them stand still in room temperature for an additional hour. The AC was then removed by filtration using filter paper and membrane filter. The NAC and DAC concentrations in the filtrate were determined using high-performance liquid chromatography-ultra violet (HPLC-UV) analysis. These adsorption experiments were performed in triplicate for each concentration. The same procedures without AC were performed on identical samples as controls. Results were expressed as the mean ± SD.

Additionally, we carried out another adsorption study in which NAC was added into AC in the presence of APAP. After placing 2.5 g amounts of AC powder into bottles, 0.375 g of APAP (37.5 mL of 10 mg/mL APAP solution) was added to the bottles. We then incubated them in a 37°C water bath for 1 h. Then, 5% NAC solution was added at 1, 5, and 9 h after APAP was added. Namely, 7 mL of the 5% NAC solution was added into the bottles and incubated for 4 h, followed by 3.5 mL of the 5% NAC solution being added into the bottles and incubated for an additional 4 h. Then, 3.5 mL of the 5% NAC solution was added and incubated for another 4 h. We then incubated the solutions for yet an additional hour. The bottles were then allowed to stand still in room temperature for another hour. The AC was then removed from each suspension by filtration. The APAP, NAC, and DAC concentrations in the filtrate were determined using HPLC-UV analysis. The samples were performed in triplicate. The blank samples without the NAC solution were run to compare APAP adsorption with and without NAC as a control.

NAC and DAC were measured by the reverse-phase HPLC consisting of the L-7100 pump, the L-7420 UV-visible detector, and the D-2500 integrator (Hitachi High-Tech Science Corporation, Tokyo, Japan) and the Degasys DG1310 (Senshu Scientific Co., Ltd. Tokyo, Japan). Separation was performed with the Inertsil ODS-3 column (4.6 mm i.d. × 150 mm; GL Sciences Inc., Tokyo, Japan). The mobile phase consisted of 15 mM phosphate buffer (pH 4.4): acetonitrile = 90: 10 (v/v) for the APAP analysis and 12 mM ammonium formate buffer (pH 3.0): acetonitrile = 96.5: 3.5 (v/v) for the NAC and DAC analysis. At a flow rate of 1.0 mL/min, the eluate was monitored for absorbance of APAP at 254 nm and the same for both NAC and DAC at 210 nm. The retention time of APAP was 5.8 min, and that of NAC and DAC was 6.9 and 21.0 min, respectively.

Mass spectrometry of NAC and DAC were measured with a JEOL AccuTOF LC-Plus T100LP mass spectrometer equipped with an ESI (electrospray ionization) interface (JEOL, Tokyo, Japan). HPLC experiments were performed on Agilent1200 system using Shiseido Capcell Core ADME (2.1 mm i.d. × 100 mm; Shiseido, Tokyo, Japan) at 40°C. The mobile phase consisted of 0.1% formic acid: acetonitrile containing 0.1% formic acid = 95: 5 (v/v) was used at a flow rate of 0.3 mL/min for isocratic separation. The retention time of NAC and DAC was 2.1 and 4.3 min, respectively.

The amount of adsorbed NAC was calculated as the amount of decreased NAC which was calculated by the difference of free NAC between the control and the sample. The amount of NAC adsorbed per gram of AC (Q) was obtained by dividing the amount of decreased NAC by the quantity of AC (1). So that we estimated the maximum adsorption capacity (Qm), using the Langmuir adsorption isotherm in the following equation (2):

$$Q = \frac{C_0 \times V_0 - C_f \times V_f}{W} \quad \cdots\cdot (1)$$

$$\frac{C_f}{Q} = \frac{C_f}{Q_m} + \frac{1}{Q_m \cdot K} \quad \cdots\cdot (2)$$

where Q (mg/g) is the amount of NAC adsorbed per gram and Qm (mg/g) is the maximum quantity of NAC adsorbed per gram of AC. C_0 and C_f (mg/mL) are the NAC concentration of the control and sample in the liquid phase at equilibrium, respectively. V_0 and V_f (mL) are the volume of the control and sample, respectively. W (g) is the quantity of AC. K (mL/mg) is equilibrium adsorption constant.

RESULTS

The adsorption isotherm is shown in Fig. 1. The adsorption of NAC onto the AC was saturated at both pHs. We then calculated the Qm to evaluate how much NAC the AC could adsorb. The Qm (apparent) in SGF was 400.00 mg/g, and that in SIF was 714.29 mg/g. Interestingly, two peaks at 6.9 min and 21.0 min appeared on the chromatogram of the sample solution using HPLC-UV (Fig. 2A), while the second peak was small in the control solution (Fig. 2B). We then performed liquid chromatography/mass spectrometry (LC/MS) analyses of the samples and identified the second peak as DAC, which was the disulfide form of NAC (NAC *m/z* 164.041 $[M + H]^+$, *m/z* 162.055 $[M - H]^-$, DAC *m/z* 325.059 $[M + H]^+$, *m/z* 323.104 $[M - H]^-$). We then simultaneously determined the concentrations of NAC and DAC. Because it was revealed that the amount of the decreased NAC, including the amount of adsorption onto AC, and the amount of conversion to DAC, we then recalculated the actual amount of adsorption by subtracting the amount of conversion to DAC from the amount of decreased NAC. We treated DAC as an NAC equivalent in the present study (NAC equivalent = DAC × 1.00617). Based on the actual amount of adsorption, the Qm (actual) in SGF was 294.12 mg/g, and that in SIF was 59.17 mg/g (Table 1).

We then evaluated whether or not the adsorption of APAP and NAC onto AC competed with each other. Because the gastrointestinal transit time of AC was about 7 h,[11] NAC was added to the AC and APAP mixed suspension in 3 parts corresponding to the loading dose and 2 times of the maintenance doses. The adsorption rate of APAP with NAC in SGF was ≥97.88%, and that in SIF was 99.00%. That of APAP with-

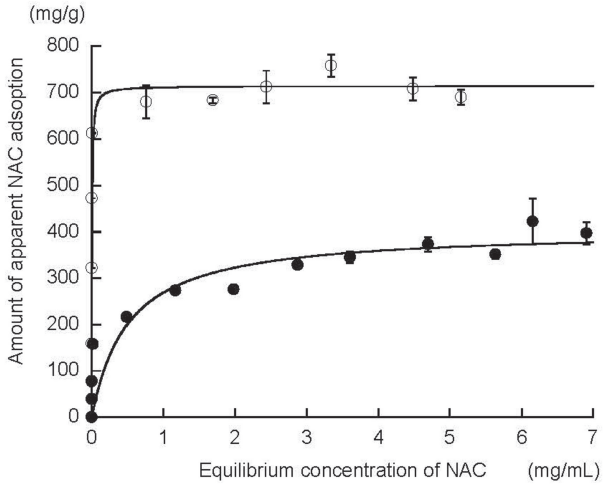

Fig. 1. Langmuir Isotherm for Apparent Adsorption of NAC onto AC in SGF (●) and SIF (○)

Adsorption isotherm data was calculated using the concentration of NAC in the control and the equilibrium concentration of NAC in a sample after the adsorption experiment. The concentration of NAC was measured using HPLC-UV. Initial concentration: 0.25–10 mg/mL, Solvent volume: 20 mL, AC: 125 mg, Temperature: 37°C, pH in solution: 1.2 (SGF) and 6.8 (SIF). The data are expressed as the mean ± SD (n = 3).

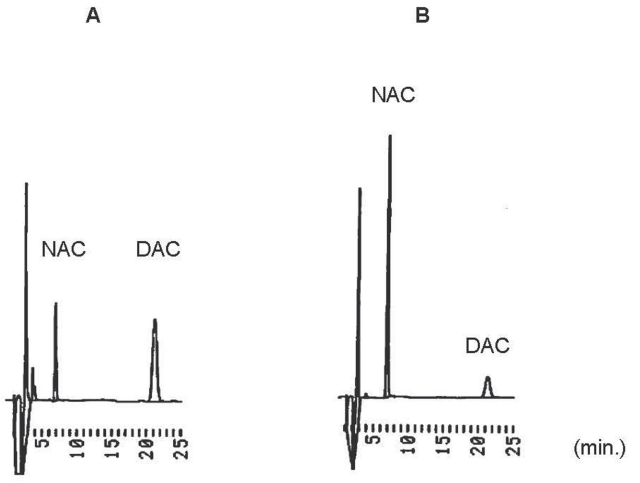

Fig. 2. Chromatograms of NAC Solution after AC Adsorption Experiment Using HPLC-UV

(A) NAC solution after AC adsorption experiment (B) NAC solution without AC (control). The retention times of NAC and DAC are 6.9 min and 21.0 min, respectively. Initial concentration of NAC: 8 mg/mL, Solvent volume: 20 mL, AC: 125 mg, Temperature: 37°C, pH in solution: 6.8.

out NAC was ≥99.95% in both medias. We then calculated the residual rate of NAC, the conversion rate to DAC and the adsorption rate onto AC after AC adsorbed APAP. The residual rate of NAC in SGF was 2.09 ± 2.07% and that in SIF was 0.25 ± 0.43%. The conversion rate to DAC and the adsorption rate onto AC in SGF were 30.28 ± 2.08% and 67.64 ± 0.31%, respectively. On the other hand, those in SIF were 90.35 ± 2.92% and 9.40 ± 2.67%, respectively (Fig. 3).

DISCUSSION

AC can adsorb a variety of drugs and toxins. However, it may also adsorb oral antidotes if the antidotes are administered simultaneously or soon after the AC is given. We evaluated the adsorption of NAC onto AC *in vitro*. We first calculated the maximum adsorption amount of NAC by the Langmuir isotherm (Fig. 1). The *Qm* was higher in SIF. It was different from what we expected in that the *Qm* was higher in SGF. Because AC has a nonpolar and hydrophobic surface, it is more likely to adsorb the nonionized form than the ionized form.[13] We estimated the rate of the nonionized form of NAC in gastric and intestinal fluids. NAC has two functional groups, which are carboxylic acid and thiol. The pKa_1 (carboxylic acid) and pKa_2 (thiol) are 3.20 and 9.62, respectively. The percentage of nonionized form of NAC in SGF (pH 1.2) is 99.0% and that in SIF (pH 6.8) is 0.03%. Thus, we predicted that NAC would be more adsorbed in SGF.

It is well known that thiols such as GSH, cysteine and NAC have antioxidant effects and high reactivity. It is also known that they are easily converted to the disulfide form. We observed a second peak on the chromatogram in samples using HPLC-UV and proved that the peak indicated the disulfide form of NAC using LC/MS (Fig. 2). Because the amount of DAC in the samples was significantly increased, we recalculated the amount of actual adsorption. The *Qm* (actual) in SGF was 294.12 mg/g, and that in SIF was 59.17 mg/g (Table 1). This proved that AC adsorption is greater for the nonionized form of NAC. Therefore, the experiment concluded as expected.

NAC was significantly oxidized if AC was presented, especially in SIF. The oxidation may occur by either or both

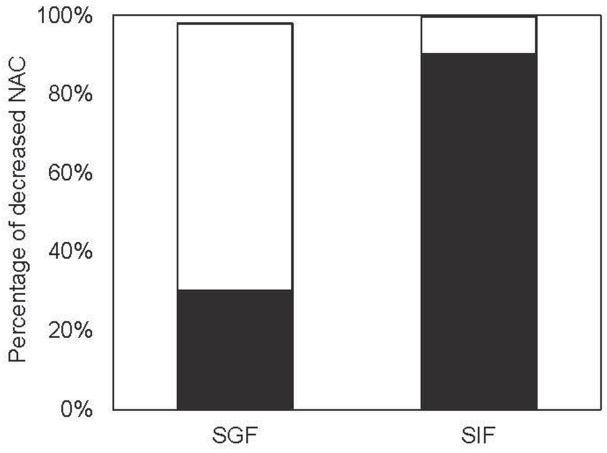

Fig. 3. The Conversion Rate to DAC and the Adsorption Rate on AC of NAC after AC Adsorbed APAP

The conversion rate to DAC (■) and the adsorption rate (□) is 30.28% and 67.64% in SGF, 90.35% and 9.40% in SIF. APAP: 0.375 g, AC: 2.5 g, NAC (total): 0.7 g, Temperature: 37°C, pH in solution: 1.2 (SGF) and 6.8 (SIF). The data are expressed as the mean (n = 3).

Table 1. Langmuir Parameters for the Adsorption of NAC onto AC

pH	Apparent adsorption			Actual adsorption		
	Qm (mg/g)	K (mL/mg)	*r*	*Qm* (mg/g)	K (mL/mg)	*r*
1.2	400.00	3.13	0.990	294.12	17.00	0.995
6.8	714.29	233.43	0.999	59.17	18.83	0.665

The values of *Qm* and K were obtained from the Langmuir equation. The *Qm* and K are maximum adsorption capacity (mg/g) and the equilibrium adsorption constant (mL/mg), respectively. Apparent adsorption was calculated from the amount of decreased NAC, and actual adsorption was calculated from the amount of adsorption by subtracting the amount of conversion to DAC from the amount of decreased NAC.

of two mechanisms, one of which was affected by pH. In an alkaline condition, thiolate anions are generated more. Thiols exhibited dose loss of protons in an alkaline condition, followed by oxidation to a free radical and radical coupling.[14] Thus, the conversion rate to DAC was increased more in SIF. Another mechanism was via an oxidation catalysis. Hayashi *et al.* showed that oxidative conversion of aliphatic and aromatic thiols to disulfides was promoted by AC.[15] However, this mechanism remains unclear. The surface of AC has a few functional groups, such as carbonyl, carboxyl, phenolic hydroxyl, lactone, and quinone groups though AC consists almost entirely of carbon. These functional groups could catalyze the oxidation of NAC. Pereira *et al.* reported a good correlation between the catalytic activity and the concentration of carbonyl/quinone groups on the surface of AC.[16] In addition, AC contains heavy metals, and its content does not exceed 50 ppm as defined by the JP XVII. It is known that several heavy metals catalyze thiol oxidation.[14] Hence, the metals contained in the AC may act as a catalyst. The oxidation of NAC may occur in higher pH and by these catalytic mechanisms.

We showed the apparent and actual *Qm*. Chinouth *et al.* showed the *Qm* in SIF at pH 7.5 was 746.9 mg/g.[17] Rybolt *et al.* also reported that the *Qm* in SGF was 243.2 mg/g, and that in SIF at pH 7.0 was 643.0 mg/g.[18] Although these results have not taken in the consideration of the conversion to DAC, they do support our apparent adsorption data. Based on the decreased amount of NAC in our study, the 50-g standard treatment dose of AC would decrease NAC by between 20 g and 35 g. This suggested that AC affected not only the loading dose but also the maintenance doses of NAC.

In the treatment of APAP poisoning (toxic dose ≥7.5 g), AC is first administered (treatment dose is 50 g), followed by NAC administered according to a 72-h oral regimen (a loading dose of 140 mg/kg, followed by 70 mg/kg every 4 h, for an additional 17 doses) at least 1 h after AC is given. We then evaluated whether or not the adsorption of APAP and NAC onto AC competed with each other. Because the gastrointestinal transit time of AC with a cathartic was within about 7 h,[11] we added NAC up to the second maintenance dose in the present study. We conducted the adsorption study in one twentieth dose of clinical situation. The adsorption rate of APAP with and without NAC was ≥97.88% in both SGF and SIF. NAC did not affect the APAP adsorption onto AC. We then determined the NAC concentration. The residual rate of NAC in SGF was 2.09% and that in SIF was 0.25%. The cause of reduction was mainly the conversion to DAC in SIF (Fig. 3). In either case, the loading and maintenance doses of NAC was almost completely decreased both in SGF and SIF even if the AC adsorbed APAP.

Klein-Schwartz and Oderda reported *in vitro* adsorption experiments.[19] When 1 g of NAC and 6 g of AC (equate 10 g of NAC and 60 g of AC) were mixed for 5 min, the average adsorption rate was 96.2%. We acquired the same results as those with the reduction rate. Ekins *et al.* compared the level of NAC in 19 volunteers who were administered NAC alone and with both AC and NAC. When both AC and NAC were administered, AUC and C_{max} of NAC were reduced by 39% and 29%, respectively.[20] Chamberlain *et al.* evaluated high doses of NAC in a volunteer study.[21] Ten healthy adults were administered 3 g of APAP and 1 h later 60 g of AC and 235 mg/kg of NAC. The AUC of NAC was increased by 38% compared with those of subjects who had been administered with 140 mg/kg doses of NAC alone. Thus, it could not be expected that NAC prevent the liver injury caused by APAP if AC stays in the gastrointestinal gut and contacts NAC. However, high doses of NAC with AC treatment would improve the bioavailability.

It was unclear that AC affected the outcomes of APAP overdosed patients treated with the 72-h oral NAC because Rumack and Peterson excluded patients given AC so as to avoid the decreasing absorption of NAC.[8] Spiller *et al.* compared the outcomes of APAP overdosed patients who received both AC and NAC or NAC alone.[22] In their study, the treatment group with both AC and NAC decreased the odds ratio (0.15, 0.03–0.59) of the frequency of elevated AST (>125 IU/L) compared with the treatment group with NAC alone. Spiller and Sawyer also compared the outcomes between the treatment group of both AC and NAC and the treatment group with NAC alone using the TESS (Toxic Exposure Surveillance System) database (1993–2004) of the United States Addiction Control Center for APAP overdosed patients.[23] The odds ratios of morbidity and mortality, AST/ALT, hypoglycemia, prolonged prothrombin time, increased creatinine, renal failure, oliguria/anuria were significantly reduced in the treatment group with both AC and NAC, compared with the treatment group with NAC alone. Spiller *et al.* concluded that the use of AC, in addition to NAC therapy, may provide improved patient outcomes.[22,23]

It is better that both AC and NAC are administered for APAP poisoning to improve the outcomes. It is also important that each agent is administered early so as to be more effective. But the effect of AC on NAC could not be mistaken based on the *in vitro* and *in vivo* studies. From our results, AC would affect the loading dose and maintenance doses of NAC. Oral NAC in a total dose of 1,330 mg/kg over 72 h is the only current regimen in Japan. Hence, the regimen should be modified to a higher dose and a shorter protocol so that the effect of AC on NAC is minimized. Intravenous formulas of NAC are available in the United States and Europe.[24] The total dosage and the term of IV NAC protocol is 300 mg/kg over 21 h. Because the bioavailability of NAC is about 11.6%,[25] the total dosage of IV NAC corresponds to 2,600 mg/kg of oral NAC. Although the saturation of absorption is unclear, the oral regimen can potentially be improved as a high dose and short-term regimen if the patient is tolerant. The shorter regimen has the benefit of a shorter hospital stay and resultant lower cost.

We evaluated the adsorption of NAC onto AC after APAP was adsorbed. The present study suggests that AC decreases the amount of NAC by adsorption and the oxidation catalytic effect. Oxidation of NAC occurred especially in SIF. Overall, if AC stays in the stomach and intestines, it may reduce the effect of both the loading and maintenance doses of NAC. Therefore, a cathartic should always be administered with AC therapy so as to enhance the elimination of the AC. In addition, the higher loading and maintenance doses should be given at least until AC is detected in the stool. Based on the IV protocol of 300 mg/kg over a 21-h period, the oral dose of NAC could be increased up to a total of 2,600 mg/kg, and the term could be shortened down to 21 h if the patient is tolerant. To combat what could otherwise be a fatal toxic drug overdose, these results suggest that the pharmacological clinical application of this methodology would allow shorter therapeutic regimens, shorter stays in hospitals, and significantly improve patient outcomes.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) Chyka PA, Seger D. Position statement: single-dose activated charcoal. American Academy of Clinical Toxicology; European Association of Poisons Centres and Clinical Toxicologists. *J. Toxicol. Clin. Toxicol.*, **35**, 721–741 (1997).
2) Yeates PJA, Thomas SHL. Effectiveness of delayed activated charcoal administration in simulated paracetamol (acetaminophen) overdose. *Br. J. Clin. Pharmacol.*, **49**, 11–14 (2000).
3) Dordoni B, Willson RA, Thompson RP, Williams R. Reduction of absorption of paracetamol by activated charcoal and cholestyramine: a possible therapeutic measure. *BMJ*, **3**, 86–87 (1973).
4) Levy G, Houston JB. Effect of activated charcoal on acetaminophen absorption. *Pediatrics*, **58**, 432–435 (1976).
5) Bainbridge CA, Kelly EL, Walking WD. In vitro adsorption of acetaminophen onto activated charcoal. *J. Pharm. Sci.*, **66**, 480–483 (1977).
6) Rumack BH, Matthew H. Acetaminophen poisoning and toxicity. *Pediatrics*, **55**, 871–876 (1975).
7) Larson AM. Acetaminophen Hepatotoxicity. *Clin. Liver Dis.*, **11**, 525–548 (2007).
8) Rumack BH, Peterson RG. Acetaminophen overdose: incidence, diagnosis, and management in 416 patients. *Pediatrics*, **62**, 898–903 (1978).
9) Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). *N. Engl. J. Med.*, **319**, 1557–1562 (1988).
10) Ayumi Pharmaceutical Corporation. Acetylcysteine solution 17.6% [AYUMI] package insert. 9th ed. (2016)
11) Neuvonen PJ, Olkkola KT. Effect of purgatives on antidotal efficacy of oral activated charcoal. *Hum. Toxicol.*, **5**, 255–263 (1986).
12) Orisakwe OE, Afonne OJ, Ilondu NA, Obi E, Ufearo CS, Agbasi PU, Maduka SO, Orish CN, Orish VN. Influence of prokinetics on the gastrointestinal transit and residence times of activated charcoal. *J. Pak. Med. Assoc.*, **52**, 354–356 (2002).
13) Cooney DO. Activated charcoal in medical applications. Informa Healthcare, New York (1995).
14) Smith MB, March J. March's advanced organic chemistry: reactions, mechanisms, and structure. 6th, Wiley-Interscience, New Jersey, pp. 1785–1786 (2007).
15) Hayashi M, Okunaga K, Nishida S, Kawamura K, Eda K. Oxidative transformation of thiols to disulfides promoted by activated carbon-air system. *Tetrahedron Lett.*, **51**, 6734–6736 (2010).
16) Pereira MFR, Órfão JJM, Figueiredo JL. Oxidative dehydrogenation of ethylbenzene on activated carbon catalysts. I. Influence of surface chemical groups. *Appl. Catal. A.*, **184**, 153–160 (1999).
17) Chinouth RW, Czajka PA, Peterson RG. N-acetylcysteine adsorption by activated charcoal. *Vet. Hum. Toxicol.*, **22**, 392–394 (1980).
18) Rybolt TR, Burrell DE, Shults JM, Kelley AK. In vitro coadsorption of acetaminophen and N-acetylcysteine onto activated carbon powder. *J. Pharm. Sci.*, **75**, 904–906 (1986).
19) Klein-Schwartz W, Oderda GM. Adsorption of oral antidotes for acetaminophen poisoning (methionine and n-acetylcysteine) by activated charcoal. *Clin. Toxicol.*, **18**, 283–290 (1981).
20) Ekins BR, Ford DC, Thompson MI, Bridges RR, Rollins DE, Jenkins RD. The effect of activated charcoal on N-acetylcysteine absorption in normal subjects. *Am. J. Emerg. Med.*, **5**, 483–487 (1987).
21) Chamberlain JM, Gorman RL, Oderda GM, Klein-Schwartz W, Klein BL. Use of activated charcoal in a simulated poisoning with acetaminophen: a new loading dose for *N*-acetylcysteine? *Ann. Emerg. Med.*, **22**, 1398–1402 (1993).
22) Spiller HA, Krenzelok EP, Grande GA, Safir EF, Diamond JJ. A prospective evaluation of the effect of activated charcoal before oral N-acetylcysteine in acetaminophen overdose. *Ann. Emerg. Med.*, **23**, 519–523 (1994).
23) Spiller HA, Sawyer TS. Impact of activated charcoal after acute acetaminophen overdoses treated with N-acetylcysteine. *J. Emerg. Med.*, **33**, 141–144 (2007).
24) Cumberland Pharmaceuticals Inc. Acetadote package insert. (2019).
25) Borgström L, Kågedal B. Dose dependent pharmacokinetics of N-acetylcysteine after oral dosing to man. *Biopharm. Drug Dispos.*, **11**, 131–136 (1990).