Evaluation of active carbon fibers used in cell biocompatibility and rat cystitis treatment

Ming-Chien Hung a,*, Sheau-Yun Yuan b,d, Shih I. Chang a, Jiunn-Wang Liao c
Tse-Hao Ko a,*, Chen-Li Cheng b,*

a Carbon Lab, Department of Materials Science and Engineering, Feng Chia University, Taichung 40724, Taiwan, ROC
b Division of Urology, Taichung Veterans General Hospital, Taichung 40705, Taiwan, ROC
c Graduate Institute of Veterinary Pathobiology, National Chung-Hsing University, Taichung 40722, Taiwan, ROC
d Department of Nursing, HungKung University, Taichung 43302, Taiwan, ROC

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ABSTRACT
Escherichia coli is the predominant pathogen in catheter-associated urinary tract infection (CAUTI) and cystitis. CAUTI treated with prophylactic antibiotics induces antibiotic resistance in hospitals and nursing homes. Therefore, we developed a physical method to treat cystitis by using activated carbon (AC) fibers to remove lipopolysaccharide (LPS) from the bladder. An in vitro assay showed that AC had remarkable LPS adsorption capability (20.24 EU per mg AC for 60 min) and good biocompatibility, as revealed by morphological observation through field-emission scanning electron microscopy and MTS assay in SV-HUC-1 cell line. In an in vivo assay in rats, 3 groups were formed: control (pyrogen-free saline), LPS, and AC (n = 10, each group). The LPS and AC groups were pretreated with protamine sulfate (10 mg/mL), and E. coli LPS (5 mg/kg) was administered to induce cystitis. Subsequently, the AC group received 0.4 mg/kg AC suspension. After 24 h, histologic analysis revealed increased urinary bladder weight (edema) and marked increase in vascular congestion and hemorrhage in the LPS group. In contrast, the AC group showed significant reduction in degree of edema, inflammatory cell infiltration, and hemorrhage (p < 0.05). Thus, AC has the potential to act as an auxiliary agent for cystitis amelioration.

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1. Introduction
Urinary tract infection (UTI) affects millions of people each year. The susceptibility to UTI is influenced by both anatomic factors as well as behavioral and physiological factors such as genetic inheritance, old age, pregnancy, diabetes, neurologic disease, urogenital dysfunction, previous pelvic surgery, and estrogen deficiency (ovariectomized and menopausal women) [1,2]. However, catheterization of the urinary tract predisposes patients to catheter-associated UTI (CAUTI), which accounts for more than 1 million cases in hospitals and nursing homes annually and approximately 40% of nosocomial infections [3]. In some patients with Parkinson disease, cortical strokes, brain tumors, normal pressure hydrocephalus, traumatic brain injury, Alzheimer-type dementia, and suprasacral spinal cord injury [4] who have dysfunction of the lower urinary tract and neurologic transmission (neurologic disorder), there is a loss of coordination between the bladder and its outlet, creating an obstruction [4]. Patients with bladder neck outflow obstruction due to benign or malignant prostatic disease [5] require long-term use of indwelling urethral (Foley) or
suprapubic catheters. The only commonly available management techniques for neurogenic bladder dysfunction or bladder obstruction might lead to frequent UTI or sepsis [6], predisposition to chronic renal inflammation, chronic pyelonephritis, renal failure, and dialysis [7], as well as increase the risks of invasive and potentially lethal bladder cancer [8]. Furthermore, long-term treatment with antibiotics can result in bladder infection by resistant organisms. In one clinical study, Escherichia coli was shown to cause 80–85% of acute episodes of uncomplicated cystitis. Staphylococcus saprophyticus and other such organisms are responsible for most of the non-E. coli episodes [9].

Systemic bacterial lipopolysaccharide (LPS)—the cell wall component of gram-negative bacteria, including E. coli—can induce many of the host defenses required for killing bacteria and up-regulates nitric oxide in RAW 264.7 macrophages [10] and cytokine production in epithelial and stromal cells [11] and whole animal models [12]. LPS has been shown to induce bladder inflammation, endotoxic shock, and multiple organ failure [13]. For example, LPS from E. coli O127:B8 induced significant bladder injury [14]. In order to establish a bladder injury model that would mimic a UTI, we adapted the procedure of Stein et al. [14] in which protamine sulfate administration is followed by LPS instilled into the bladder to produce inflammation [14].

In recent decades, activated carbon (AC) has not only been widely used in separation, purification, and catalytic processes because of its high specific surface area, high adsorption capacity, highly porous structure, and special surface reactivity [15] but also used for medicinal purposes such as removal of toxins from the stomach [16,17] and inflammatory cytokines in the blood [18,19], in kidney diseases [20] and infected wounds [21], and to reduce odor and uremic toxins in patients with chronic kidney disease.

Following the advancement in carbon material technology, a newer fabric-like AC has been processed through carbonization and activation of organic fibers. AC fibers show faster adsorption kinetics than pelletized or granular AC. Because AC fibers have micropores unlike granular AC, they have higher adsorption capacity and shorter adsorption time than granular AC.

Because of its good adsorptive properties, AC was used as a therapeutic modality for the treatment of cystitis, considering that it might serve as a feasible alternative to antibiotics. Several studies have shown that AC can be applied in the adsorption of high-molecular-weight toxic substances such as proteins and LPS in the treatment of sepsis or multi-organ failure [22]. George and Davies reported that AC cloth is a suitable material for bacterial removal in medical applications [23] and reported that 95.3% of bacteria (E. coli) were adsorbed by AC cloth in 30 min. Therefore, we adopted a new strategy, where in an adsorption material was instilled into the bladders of rats to “catch” bacteria or LPS and remove them from the bladder (Fig. 1). In this study, we investigated physical and chemical properties of AC, including the endotoxin-binding capability and in vitro biocompatibilities. Finally, we conducted an in vivo study of LPS-induced cystitis model in rats to evaluate the efficacy of AC for the treatment of LPS-induced cystitis.

2. Experimental

2.1. Materials and animals

The polyacrylonitrile (PAN)-based AC fiber fabric was purchased from Taiwan Carbon Technology Co., Ltd. (Taichung, Taiwan). The specific surface area of AC was 1,023 m²/g as per the Brunauer, Emmett, and Teller (BET) method. Animal experiments were performed on adult female Sprague–Dawley rats (250 ± 50 g) housed in individual cages containing sawdust bedding, and food and water was provided ad libitum; the animal house was maintained at a constant temperature of 22 °C with a 12 h alternating light–dark cycle (light phase 0700–1900 h). The experimental procedures involving the use of animals in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the Taichung Veterans General Hospital.

2.2. Sample preparation

AC was ground into powder by using a mortar and pestle and mixed with sterile water to remove impurities. A polytetrafluoroethylene (PTFE) membrane filter was used to collect AC powder and remove water. The powder was then placed into a glass dish, covered with an aluminum foil, and then sterilized in an autoclave. AC powder was reconstituted with sterile normal saline to a final concentration of 0.4 mg/mL.

2.3. LPS binding analysis

The endotoxin (LPS) binding capacity of AC was investigated using a standard E. coli endotoxin O55:B5 (Kinetic-QCL 2400 Test Kit No. 50-650H [50-650H; Lonza, USA]). Before the experiment, the control group (blank) and experimental group (3 mg of AC) were placed in two 10 mL solid screw clear vials (with PTFE silicone septum), which were previously autoclaved at 15 psi for 30 min by using an electric pressure steam sterilizer (Model No. 25X; Wisconsin Aluminum Foundry Co., Inc., WI, USA), followed by heating in an oven (Model 16; Precision Scientific Co., IL, USA) at 210 °C for 3 h for sterilization.

After steam sterilization, the experimental and control groups were incubated with 81 EU of E. coli endotoxin at 37 °C, and sample aliquots were removed at 0, 15, 30, and 60 min. All solutions were prepared by using endotoxin-free water (LAL reagent water; W50-640, Lonza Walkersville Inc., USA). All solution transfers were performed using endotoxin-free devices. Sterile, disposable plastic ware was used at all times to prevent endotoxin contamination.

Endotoxin assay was then performed using a quantitative kinetic chromogenic LAL method (Kinetic-QCL 2400 Test Kit No. 50-650H; BioWhittaker; LAL Lot No. 000271843) at 37 °C by using an automated microplate reader (ELx808 Ultra Microplate Reader; Lonza Group Ltd., Switzerland). Data from the samples were compared with the standard curve, which ranged from 0.005 to 50 EU/mL. The endotoxin concentration of the test samples was calculated by comparing the reaction time needed to reach the defined optical density (reaction time) with a standard curve generated from the log/log corre-
lation between defined endotoxin amounts and reaction time. All results for the test samples are mean values of triplicate determinations. The reaction time was determined using software WinKQCL 4.0.2.

2.4. Cell culture

SV-40-immortalized normal uroepithelial (SV-HUC-1) cells were purchased from the Food Industry Research and Development Institute (FIRDI; Hsinchu, Taiwan). The cell lines were cultured in McCoy’s 5A and RPMI medium supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, USA), L-glutamine (200 mM), and penicillin/streptomycin/amphotericin B (10,000 IU/mL and 10,000 and 25 μg/mL, respectively) solution. The cell lines were cultured in 75 cm² flasks, subcultured three times a week, and incubated in a humidity-controlled CO₂ incubator at 37°C with 5% CO₂. All the experiments were performed in a clean atmosphere.

2.5. Cell morphology

SV-HUC-1 cells were used to investigate the morphology after treatment with AC. AC powder at concentrations of 0.05, 0.1, 0.2, and 0.5 mg was placed in 24-well plates containing the cells and mixed. Each well had 1 mL cell culture medium. After 24 h, the cell morphology was observed using an optical microscope (IX71; Olympus, Japan) and by field emission-scanning electron microscopy (FE-SEM; S-4800; Hitachi, Japan).

Cells were prepared for SEM by fixation with 2% glutaraldehyde buffered in phosphate-buffered saline (PBS) and post-fixed in 1% osmium tetroxide. Next, the samples were dehydrated in graded ethanol concentrations. Samples observed with FE-SEM did not require surface coating, and an accelerating voltage of 0.7 kV was employed. Images of the samples were obtained at 1-5 k magnifications.

2.6. Cell cytotoxicity assay

SV-HUC-1 cells (2 × 10⁴) were plated onto 24-well plates and incubated in a humidified atmosphere of 5% CO₂ at 37°C for 24 h. After 24 h, AC powder was added at concentrations of 0.05, 0.1, 0.2, and 0.5 mg/mL into prepared 24-well plate cells and incubated for 24 h. A previous study has found that carbon material with large surface area can adsorb the end products of cytotoxicity assays [24]. Thus, interference in cytotoxicity data was avoided by performing MTS cytotoxicity assay for verification.

The MTS cytotoxicity assay (Cell Titer 96 AQueous Non-Radioactive Cell Assay; Promega USA) was used to evaluate cytotoxicity. MTS solution (200 μL from 1 mg/mL) was added to each well, and the plates were further incubated at 37°C for 1 h. An aliquot of 200 μL was measured using a Microplate Autoreader (Tecan Deutschland GmbH) at a wavelength of 490 nm. The experiments were carried out in triplicate.

2.7. DAPI fluorescence staining

SV-HUC-1 cells (2 × 10⁴) were plated onto 24-well plates and incubated in a humidified atmosphere of 5% CO₂ at 37°C for 24 h. After 24 h, AC powder was added to concentrations of 0, 0.05, 0.1, 0.2, and 0.4 mg/mL into prepared 24-well plate cells and incubated for 24 h. After 24 h, the cells were washed with PBS and fixed in 2% paraformaldehyde for 30 min, and then permeabilized with 0.1% Triton-X 100 in PBS for 30 min. Nuclei were stained by incubating the cells with DAPI (1 μg/mL) and examined under an Olympus IX71 fluorescence microscope.

2.8. Induction of cystitis

The following three steps of rat model of LPS-induced cystitis experiment were performed before comparison of with and without AC treatment [25]. In the first step, female rats were anesthetized with urethane (1.2 g/kg subcutaneously) and then catheterized through the urethra using a lubricated PE-50 catheter in order to evacuate the residual urine in bladders by gentle aspiration (all groups).

The second step was induction of cystitis. The rats were administered 0.5 mL protamine sulfate (10 mg/mL) instilled into the bladder and retained for 45 min; the bladders were then emptied and washed with saline. After 2 h, the bladders

Fig. 1 – Schematic representation of the treatment process for LPS-induced cystitis in rats by using AC suspension. The first AC suspension was instilled into the bladder by using a catheter for the treatment of LPS-induced cystitis in rats. After the AC binds LPS, the AC suspension was removed by urination to ameliorate cystitis (decreased edema, inflammation, and hemorrhage). (A colour version of this figure can be viewed online.)
were emptied, and the rats were then administered 0.5 mL (5 mg/kg) LPS once for 1 h (LPS group and AC group). After 1 h, the bladders were emptied, and the rats were subjected to the third treatment with 0.5 mL (0.4 mg/kg) AC once for 1 h (AC group). After the end of the last treatment, the rats were injected with gentamicin (6 mg) to decrease the chances of any subsequent infection. The catheters were then gently removed, and the animals were allowed to recover. All rats were euthanized 24 h after the last instillation. The bladders were removed and immersed in buffered formalin for morphological analysis.

2.9. **Histological evaluation of cystitis**

Twenty-four hours after cystitis was induced, the bladders were dissected. For each rat, the dissected bladder was fixed in 10% formalin and routinely processed and embedded in paraffin wax. Two-micrometer sections were stained with hematoxylin and eosin (H and E). The urinary bladders were evaluated according to the criteria described by Jerde et al. [26], which included mucosal inflammatory cell infiltration and the presence of interstitial edema and hemorrhage. Briefly, the edema in each quadrant was evaluated using a scale as follows: 0 = no edema; 1 = mild edema, not expanding the width of the submucosa; 2 = moderate edema, expanding the mucosal region less than double the normal size; and 3 = severe edema, doubling the area of the mucosal region or greater. Leukocyte infiltration in each section was evaluated using a scale as follows: 0 = no leukocyte infiltration per mm²; 1 = mild infiltration or less than 30 leukocytes found per mm²; 2 = moderate infiltration, i.e., between 30 and 60 leukocytes per mm²; and 3 = severe infiltration, or greater than 60 leukocytes present per mm². The areas of hemorrhage for each cross-section were divided by 10. If a cross-section had an area of hemorrhage greater than 9, it was considered confluent hemorrhage and scored as 10.

2.10. **Measurement of vesical edema**

Vesical vascular edema was quantified as bladder wet weight (BWW), and plasma protein extravasation was measured using the Evans blue dye leakage technique [27]. Anesthesia was induced by intraperitoneal administration of urethane (1.2 g/kg). The external jugular vein was cannulated for injecting Evans blue dye (50 mg/kg) at a dose volume of 25 mg/mL in PBS. The dye was administered 30 min before the animal was exsanguinated by infusion of 50 mL of 0.9% w/v saline into the left cardiac ventricle at 37 °C. The urinary bladder was then removed and blotted dry before weighing, and the content of dye was determined by spectrophotometry (at 630 nm) after extraction in 1 mL of DMSO at 56 °C for 24 h. Plasma protein extravasation was expressed as the content of Evans blue dye in micrograms per gram of tissue.

2.11. **Statistical analysis**

The results are reported as mean ± SEM for all data on the basis of at least 5 determinations. Values with \( p < 0.05 \) were considered as statistically significant. For histopathological data, statistical evaluation was performed using Kruskal–Wallis

| Table 1 – The specific surface area and pore parameters of AC and CF. |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Sample | \( S_{BET} \) (m²/g) | \( S_{t-Micro} \) (m²/g) | \( S_{t-Ext} \) (m²/g) | \( V_{Total} \) (cm³/g) | \( V_{Micro} \) (cm³/g) | \( V_{Meso} \) (cm³/g) | \( D_{avg} \) (nm) |
| AC | 1023.3 | 567.8 | 455.5 | 0.409 | 0.319 | 0.068 | 1.93 |

\( S_{BET} \) is the specific surface area calculated using the standard BET method.

\( S_{t-Micro} \) is the t-plot micropore surface area.

\( S_{t-Ext} \) is the t-plot external surface area.

\( V_{Total} \) is the total pore volume computed from adsorption at the \( P/P_0 \) value (0.99).

\( V_{Micro} \) is the volume of micropores (less than 2 nm).

\( V_{Meso} \) is the volume of mesopores (width, 2–50 nm).

\( D_{avg} \) is the average pore diameter.

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Fig. 3 – (A) The AC adsorption kinetics of endotoxin (LPS; pH 7, T = 37 °C). Initial concentration of LPS ("\(^{-}\)" represents 13.5 EU/mL). The amount of AC is 3 mg, and the total solution volume is 6 mL. (B) The time course of LPS adsorption capacities by AC were 7.85 ± 1.24, 10.63 ± 1.02, 14.4 ± 0.96, 16.09 ± 0.92, and 20.24 ± 0.62 EU/mg at 0, 15, 30, 45, and 60 min, respectively (p < 0.05). (A colour version of this figure can be viewed online.)

Fig. 4 – Morphology of SV-HUC-1 cells cultured with different concentrations of AC for 24 h, as revealed by OM. (A) No AC, (B) 0.2 mg/mL AC, (C) SV-HUC-1 cells cultured with 0.2 mg/mL AC for 72 h and morphology was observed using FE-SEM, (D) Cell tail; resolving power, 2,000×, (E) the lamellum width was 15 μm; resolving power, 3,000×, (F) cells adhering to the AC surface showed intact filopodium and lamellipodium structures; resolving power, 5,000×.

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nonparametric analysis of variance followed by Mann–Whitney U test.

3. Results and discussion

3.1. Material morphology and properties

The field emission-scanning electron microscopy (FE-SEM) was used to confirm the morphology of AC powder granules. Fig. 2A shows that a single AC granule was 10–100 μm long and 5 μm wide; this suggested that AC granules could be easily instilled into the bladder and removed from the urine. Further, as shown in Fig. 2B, AC surface had many nano-pores. These nano-pores were formed by the vapor during the activation process. The porosity of the adsorbent material plays a crucial role in physical adsorption. Thus, these pores are important in adsorption performance of AC.[28]

The relationship between pore structure and adsorption performance was investigated by analyzing the adsorption performance of AC by using a specific surface area analyzer and determining N₂ adsorption at 77 K by using the BET method. Table 1 shows that the BET specific surface area (S_{BET}) of AC was 1023 m²/g, and the adsorption average pore width was 1.93 nm. As shown in Table 1, the surface of AC was formed by micropores and contained 55.5% t-plot micropores (equal to 568 m²/g t-plot micropore surface area). This result for specific surface area and pore properties can help further clarify the relationship between AC and LPS.

3.2. In vitro study

3.2.1. LPS binding effect

Fig. 3 shows the result for the LPS-binding capability of AC. The result revealed that the measured LPS concentration was gradually decreased by AC adsorption, from initial 13.5 EU/mL to 9.58 EU/mL in the early stage near “zero” time point. The LPS-binding capability of AC in vitro was detected using kinetic methods, expressed in linearity and in a time-dependent manner. For example, the LPS concentration decreased from 8.19 EU/mL to 3.38 EU/mL at the indicated times (15, 30, 45, and 60 min) (p < 0.05). These findings suggest that

![Fig. 5 – Analysis of the effect of AC on apoptosis in SV-HUC1 cells. After 24 h, samples cultured with different AC concentrations (0, 0.1, 0.2, and 0.4 mg/mL) in DMEM medium were collected and subjected to DAPI staining (blue); they showed intact nuclei. (A colour version of this figure can be viewed online.)](image-url)
AC had the maximum binding capability to endotoxin at an adsorption concentration of 20.24 EU/mg at 60 min. According to a previous study [29], per gram bone char (BC) (a kind of active carbon, BET = 130 m²/g) had 29 EU/g maximum binding capability to endotoxin at 6 h when equilibrium conditions were achieved. AC showed a 698 times better binding capability than BC. The major reason for this was AC has a large surface area. Therefore, AC had remarkable and rapid endotoxin adsorption ability. The above result showed that the maximum adsorption quantity of AC was 20.24 EU LPS per milligram of AC at 37 °C and pH 7.0. This suggested that AC has good endotoxin-binding ability.

3.2.2. Cell morphology and biocompatibility analysis
Fig. 4 shows the results of the morphological analysis of SV-HUC-1 cells. The SV-HUC-1 cells were divided into the normal group (cells cultured without AC) and experimental group (cells cultured with 0.2 mg/mL AC). Optical microscope (OM) observations at 200x or 400x revealed that, after 24 h, the cells of the normal group had a large amount of squamous urothelial epithelium cells that were adherent and formed a monolayer (Fig. 4A), while those of the experimental group had better cell viability at 24 and 72 h (Fig. 4B and C). Further, morphological analysis using FE-SEM revealed that SV-HUC-1 urothelial epithelium cells adhered to AC surfaces and divided by protruding tails during the 24 h incubation (Fig. 4D). After 72 h, the motile cells had the broadest and widest lamellum (diameter, 10–15 μm; Fig. 4E and F). The high-resolution images (Fig. 4E and F) show the structure of lamellipodium and filopodium (small white arrows). They commonly consist of actin filaments and are useful for maintaining the cellular environment before cells begin to migrate. This suggests that SV-HUC-1 cells can stably adhere to the surface of AC within 24–72 h.

We also used DAPI fluorescence staining to further assess the interactions between the SVHUC-1 cells and AC fiber granules. First, the human bladder epithelial SVHUC-1 cells were cultured with different AC fiber suspension concentrations (0, 0.1, 0.2, and 0.4 mg/mL) for 24 h. Next, the DAPI fluorescence staining was used to confirm the relationship between concentration and biocompatibility. This method can allow the observation of cell apoptosis induced by AC fiber granules. Fig. 5 shows the DAPI-stained of intact nuclei of SVHUC-1 cells, as observed under a fluorescence optical microscope (FOM). This means that SVHUC-1 cells cultured with different AC fiber suspension concentrations for 24 h did not induce apoptosis. This implies that the physical form of AC shows good biocompatibility.

Thus, the cell morphology assay enabled to not only test the biocompatibility of AC but also investigate its cytotoxicity. Barnes et al. [18] reported that carbon materials with a large surface area can adsorb constituents of cell culture medium and the end products of cytotoxicity assays, such as insoluble MTT-formazan crystals, resulting in a false cytotoxic result. Therefore, we performed the MTS assay to assess the...
cytotoxicity of AC in this study. SV-HUC-1 cells were cultured in medium containing different concentrations of AC (0, 0.05, 0.1, 0.2, and 0.5 mg/mL) to confirm the biocompatibility between the cells and AC material. After 24 h, the cell survival rate was more than 85% (compared to that of the control, \( p < 0.05 \)), as determined by MTS (Fig. 6).

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**Fig. 8 – Histopathological findings of LPS-induced cystitis in rats.** (A) and (B) normal structure of the urinary bladder in a normal female rat. (C) and (D) LPS (5 mg/kg) induced urothelial cell injury. Note: extensive leukocyte infiltration (arrowheads) in the bladder submucosa; edema shows greater urothelium separation from the detrusor muscle within the bladder submucosa of rats of the control group compared to that in the AC group. (E) and (F) the bladders are treated with LPS for an hour and then administered AC (0.4 mg/kg). (H and E; A, C, E, 100× and B, D, F, 400×). (U = urothelium, D = detrusor muscle). (A colour version of this figure can be viewed online.)

**Table 2 – Histological analysis of the effect of AC on LPS-induced hemorrhagic cystitis.**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Edema</th>
<th>Leukocytes infiltration</th>
<th>Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.50 ± 0.27</td>
<td>0.38 ± 0.18</td>
<td>0.75 ± 0.41</td>
</tr>
<tr>
<td>LPS</td>
<td>2.50 ± 0.27(^a)</td>
<td>2.50 ± 0.27(^a)</td>
<td>4.63 ± 0.41(^a)</td>
</tr>
<tr>
<td>LPS + AC</td>
<td>1.63 ± 0.26(^b, (34%))</td>
<td>1.25 ± 0.16(^b, (50%))</td>
<td>1.63 ± 0.32(^b, (64.7%))</td>
</tr>
</tbody>
</table>

Control group was saline alone; LPS, (5 mg/kg) lipopolysaccharide; LPS + AC, after treatment with LPS for 1 h, AC was instilled at 0.4 mg/kg. (%) indicated \([\text{LPS} - (\text{LPS + AC})]/\text{LPS} × 100\%\).

\(^a\) \( p < 0.05 \) LPS or LPS + AC compared with the control group.

\(^b\) \( p < 0.05 \) LPS + AC group compared with LPS alone. Values are means ± SEM. \( n = 10 \) rats in each group.

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3.3. In vivo study

In the in vivo study, the hemorrhage cystitis animal model reported by Stein et al. was used [14]. This model might suggest whether AC instillation can significantly inhibit LPS-induced cystitis as revealed by the inflammation indices, i.e., bladder edema, leukocyte infiltration, and hemorrhage indices.

3.3.1. Vesical vascular edema

In the control (saline) group, the BWWs of the control, LPS, and AC groups were 42, 60, and 47 mg/100 g body weight, respectively. The BWWs of the LPS group were significantly higher than those of the control group. Similarly, the LPS group had a significantly higher BWWs than those of the AC groups ($p < 0.05$), as shown in Fig. 7A.

Furthermore, LPS induced extravasation of Evans blue in the bladder (9.83 ± 0.27 g/g bladder tissue, 90.5% increase, $p < 0.05$) compared with that in the control group (0.93 ± 0.12 g/g bladder tissue). AC treatment significantly reduced the LPS-induced extravasation of Evans blue by 65.2% as shown in Fig. 7B.

The results showed that AC (0.4 mg/kg) treatment decreased BWW and Evans blue extravasation in the bladder (the parameter used to detect vascular permeability) induced by LPS (5 mg/kg).

3.3.2. Histopathological evaluation

Histologic analysis showed that LPS induced not only macroscopic urinary bladder weight increase (edema) and a marked increase in vascular congestion and hemorrhage but also microscopic alterations such as mucosal sloughing, edematous submucosa, neutrophil infiltration, lymphocyte infiltration, RBC extravasation, and vessel thrombosis (arterial or venous) in the rats (Fig. 8). The histological scores were edema (2.5), leukocytes infiltration (2.5), and hemorrhage (4.63). In contrast, 0.4 mg/kg AC significantly decreased the degree of edema (1.63), inflammatory cell infiltration (1.25), and hemorrhage (1.63) in the animals ($p < 0.05$). The percentage of each investigated sample was significantly decreased by 34%, 50%, and 64.7%, respectively (Fig 8 and Table 2). Thus, the in vivo study indicated that AC can decrease the BWW, Evan’s blue extravasation (vesicular permeability), submucosa edema, neutrophil infiltration, and hemorrhage, which were caused by LPS. AC might therefore have a potential as an alternative therapeutic treatment for clinical bladder disease.

4. Conclusions

In this study, fabric-like AC was applied as a carbon-based adsorption material. The result showed that AC has a high specific surface area (1023 m$^2$/g) and micropore structure (approximately 55.5%). AC was also found to have an excellent LPS-binding performance. The result confirmed that AC can rapidly bind endotoxin (20.24 EU/mg) at 60 min. Thus, this study showed that AC can be effectively used as an adsorbent material.

The in vitro biocompatibility test was performed using SV-HUC-1 cells incubated with AC. The MTS and DAPI fluorescence staining results confirmed that AC has low cytotoxicity and can thus be used for in vivo studies in rats. Finally, the in vivo study indicated the protective effect of AC on experimental LPS-induced cystitis in female rats. AC successfully alleviated the inflammation indices, i.e., edema, leukocytes infiltration, and hemorrhage, as revealed by the histopathological assay, and attenuated the BWW increase and vascular permeability. Thus, AC appeared to decrease LPS-induced cystitis in female rats. Therefore, AC, which has a short treatment time, has a potential to be used as an alternative therapeutic treatment for clinical bladder disease or as an auxiliary antibiotic treatment to help cystitis patients.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contribution

Ming-Chean Hung: Guarantor of integrity of the entire study; study design/concept; data acquisition/analysis/interpretation; manuscript drafting/revision; literature search; experimental procedures; statistical analysis.

Sheau-Yun Yuan: study design/concept; data acquisition/analysis/interpretation; manuscript drafting/revision; statistical analysis.

Shih I. Chang: experimental design/execution; data acquisition/analysis/interpretation; experimental procedures.

Jiunn-Wang Liao: sample collection, specimen production, hematoxylin and eosin staining, image analysis/data acquisition; statistical analysis.

Chen-Li Cheng: study design/concept; literature search; data analysis/interpretation; manuscript drafting/revision.

Tse-Hao Ko: study design/concept; literature search; data analysis/interpretation; manuscript drafting/revision.

All the authors have approved the final version of the manuscript.

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