Scratching Behavior of ICR-Derived Glomerulonephritis (ICGN) Mice

Yohei MIYAMOTO1)*, Hideo UMEUCHI2), Takahiro KUROKAWA3), Kaoru NAKAO2) and Kiyoshi OKANO2)


(Received 30 September 2009/Accepted 13 April 2010/Published online in J-STAGE 27 April 2010)

ABSTRACT. The ICR-derived glomerulonephritis (ICGN) mouse, an inbred strain with a hereditary nephrotic syndrome, is considered a good animal model of human idiopathic nephrotic syndrome. ICGN mice show proteinuria at a young age, developing hyperlipidemia, anemia and edema later on. However, their behavior associated with pruritus due to renal dysfunction has not been sufficiently investigated. In the present study, we examined whether ICGN mice exhibit the scratching behavior reflecting pruritus. Mice aged 21 or 27 weeks were found to scratch persistently or intermittently, particularly those with scars. Furthermore, the scratching may have reflected a pruritus associated with renal dysfunction because it was inhibited by an opioid antagonist, naltrexone (3 mg/kg), effective against pruritus in hemodialysis patients. The results suggest that the ICGN mouse is a useful model with which to examine pruritus due to renal dysfunction.

KEY WORDS: ICR-derived glomerulonephritis (ICGN) mouse, naltrexone, pruritus, renal dysfunction, scratching behavior.

Itching, along with pain, is a major component of nociception and an important symptom of systemic problems, as well as skin diseases [30]. Many pruritic conditions do not originate in the skin, but are the result of systemic abnormality. The diseases that can cause pruritus include renal failure, cholestasis, Hodgkin’s lymphoma, polycythemia vera, solid tumors, and many others [10]. In patients on renal dialysis, the occurrence of pruritus increases with time and can reach 80% [11]. Uremic pruritus in hemodialysis patients is intractable, but recently an effective treatment using nalfurafine hydrochloride, a novel derivative of the opioid receptor antagonist naltrexone and a selective μ-opioid receptor agonist, has been established [16, 23]. Naltrexone also suppresses the itching sensation in patients with chronic cholestasis, chronic renal failure and atopic dermatitis [2, 14, 21]. Therefore, the μ-opioid receptor system is involved in processing of the itching sensation in the central nervous system. There are very few animal models suitable for pharmacological study of uremic pruritus. The MRL/Mp-lpr/lpr (MRL/lpr) mouse, a unique strain deficient in Fas-mediated apoptosis, develops severe autoimmune diseases, such as glomerulonephritis, polyarthritis and arteritis [5, 19, 25], but is not a specific model. The ICR-derived glomerulonephritis (ICGN) mouse, an inbred strain with a hereditary nephrotic syndrome, is considered a good model of idiopathic nephrotic syndrome [1] or nephritis [3, 7, 9]. ICGN mice show proteinuria at a young age, developing hypoproteinemia, hyperlipidemia, anemia and systemic edema later on [17, 18, 22]. In the present study, we examined whether ICGN mice exhibit scratching behavior related to renal dysfunction.

ICGN mice established via more than 30 inbred crosses at the National Institute of Infectious Diseases (NIID, Tokyo, Japan) were purchased as newborn animals from the Laboratory Animal Resource Bank at the National Institute of Biomedical Innovation (NIBIO, Osaka, Japan) and maintained by brother-sister matings at the Pharmaceutical Research Laboratories of Toray Industries, Inc. Normal ICR mice (Japan SLC Inc., Japan) were left untreated as a control. The animals were housed in groups of 5 animals in autoclaved polycarbonate cages and given a standard diet (CRF-1: Oriental Yeast, Japan) and tap water ad libitum in an air-conditioned room (temperature and humidity: 20–26°C and 40–70%, SPF) under controlled lighting conditions (12 hr light/12 hr dark). Six ICGN mice and two ICR mice were used in the first experiment, and fifteen ICGN mice were used in the second experiment. All experiments were conducted according to the Guidelines for Animal Experiments, Research & Development Division, Toray Industries, Inc.

In the first experiment, general scratching behavior was observed according to a method described previously [13]. On the day of testing, mice were individually placed in sections of the observation cage (the cage consisted of four 10 × 14 × 30H cm sections) to acclimate for about 30 min. Then, their behavior was recorded for 120 min by an unattended video camera (DIGICAM NV-GS55K, Panasonic, Japan), and the frequency of scratching for every 5 min was determined by replaying the recorded footage at 13:00–16:00. Usually, mice scratch with their paws several times a second; therefore, a series of these scratches was counted as one scratch event. In the second experiment, the effect of an opioid antagonist, naltrexone, on scratching behavior was investigated in ICGN mice. Saline was administered subcutaneously to each mouse on Day 1 as a control, and the
From these data, it is considered that serum creatinine as a means for females (n=5), and the data for ICR mice in SLC shows the other hand, background serum creatinine data of ICGN mice at 21 or 27 weeks of age scratched persistently or intermittently, scratching behavior reflecting a pruritus. ICGN mice at 21 weeks of age is shown in Table 2. Scratching was inhibited significantly by naltrexone. Naltrexone at a dose of 3 mg/kg did not completely attenuate the scratching behavior in the ICGN mice. These results suggest that the scratching behavior is likely to be sufficient for use as an antipruritics.

We examined whether the ICGN mouse exhibits the scratching behavior reflecting a pruritus. ICGN mice at 21 or 27 weeks of age scratched persistently or intermittently, while ICR mice rarely scratched. The scratching behavior was particularly remarkable among the mice with scars. On the other hand, background serum creatinine data of ICGN mice at the NIBIO show means ± SD levels of 0.40 ± 0.08 mg/dl in 20-week-old males (n=5), 0.48 ± 0.19 mg/dl in 26-week-old males (n=5) and 0.64 ± 0.23 mg/dl in 26-week-old females (n=5), and the data for ICR mice in SLC shows means ± SD levels of 0.40 ± 0.10 mg/dl (unpublished data). From these data, it is considered that serum creatinine as a renal marker elevates in some of 20-week-old over ICGN mice. These results suggest that the scratching behavior is due to progression of renal dysfunction [17, 18, 22]. In addition, the scratching behavior of ICGN mice seemed to be associated with pruritus due to renal dysfunction because it was significantly inhibited by an opioid antagonist, naltrexone, effective for pruritus in hemodialysis patients [2, 14, 21].

Table 1. The scratching behavior of ICGN and ICR mice

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Mice</th>
<th>Sex</th>
<th>Age</th>
<th>Skin scar*</th>
<th>Number of scratching events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ICR</td>
<td>Male</td>
<td>19-week</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>ICR</td>
<td>Male</td>
<td>19-week</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>ICR</td>
<td>Male</td>
<td>21-week</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>ICR</td>
<td>Male</td>
<td>21-week</td>
<td>–</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>ICR</td>
<td>Male</td>
<td>27-week</td>
<td>–</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>ICGN</td>
<td>Male</td>
<td>21-week</td>
<td>–</td>
<td>148</td>
</tr>
<tr>
<td>7</td>
<td>ICGN</td>
<td>Female</td>
<td>21-week</td>
<td>+</td>
<td>646</td>
</tr>
<tr>
<td>8</td>
<td>ICGN</td>
<td>Female</td>
<td>27-week</td>
<td>+</td>
<td>950</td>
</tr>
</tbody>
</table>

* Skin scar level: –, no; +, slight (rostral back scar); ++, severe (facial, rostral back and caudal back scar). The number of times the animal scratched itself was counted for 120 min.

number of times each animal scratched itself was counted within 15 and 120 min post-administration. Naltrexone hydrochloride (Sigma, St. Louis, MO, U.S.A.) was administered subcutaneously at a dose of 3 mg/kg to the same mice on Day 3, and the number of scratching events was counted within 15 and 120 min post-administration. Counting was performed between 13:00 and 16:00. Statistical differences between the naltrexone-treated ICGN mice and saline-treated mice in the second experiment were determined with a paired t-test. The results were considered significant at P<0.05 or P<0.01. All measured values in the second experiment are presented as means ± SEM.

The scratching behavior in the ICGN mice at 21 or 27 weeks of age and the ICR mice at 19 weeks old age is shown in Table 1. In 2 ICR mice without scars, almost no scratching was observed. Of the six ICGN mice, two animals with scars (slight scar, rostral back scar; severe scar, facial, rostral back and caudal back scar; not including edema in both) on their skin scratched persistently, and two animals without scars scratched intermittently. The effect of naltrexone on the scratching behavior of the ICGN mice at 16 to 29 weeks of age is shown in Table 2. Scratching was inhibited significantly by naltrexone. Naltrexone at a dose of 3 mg/kg did not completely attenuate the scratching behavior in the ICGN mice. On the other hand, naltrexone at doses of 3 or 10 mg/kg or other antipruritic drugs dose not completely reduce the scratching behavior in various itch models [4, 24, 25]. Therefore, experimental itch-related scratching behavior might be attenuated by antipruritic drugs, and the effect of naltrexone in ICGN mice is likely to be sufficient for use as an antipruritics.

Table 2. The effect of naltrexone on the scratching behavior of ICGN mice

<table>
<thead>
<tr>
<th>Test article</th>
<th>Number of scratching events( ^{\dagger} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(^b)</td>
<td>54.5 ± 16.5</td>
</tr>
<tr>
<td>Naltrexone(^b)</td>
<td>25.7 ± 7.9*</td>
</tr>
</tbody>
</table>

a) Saline was administered subcutaneously to each mouse on Day 1 as a control.
b) Naltrexone hydrochloride (Sigma, U.S.A.) was administered subcutaneously at a dose of 3 mg/kg to the same mice on Day 3.
c) The number of times the animal scratched itself was counted within 15 and 120 min after each administration. Values are means ± SEM (n=15). * P<0.05: significant difference at 0.05%.

Furthermore, nalfurafine produces anti-scratching behavior in various itch models such as mice injected intradermally with histamine [23], substance P [23] or compound 48/80 [28]; NC/Nga atopic dermatitis mice [15]; and MRL/lpr autoimmune disease mice [25]; and ethynylestradiol-induced cholestasis rats [6]. Based on these findings, nalfurafine is also expected to have an anti-scratching effect in ICGN mice. In conclusion, the ICGN mouse is a useful and specific model with which to examine pruritus due to renal dysfunction.
REFERENCES


