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RENAL AND HEPATIC IMPAIRMENT BROUGHT ON BY CISPLATIN WITHOUT COMPROMISING ANTICANCER EFFICACY.

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ABSTRACT

One of the main disadvantages of cisplatin's therapeutic usage is that it can cause hepatic and renal dysfunctions. Part of its toxicity's chemical mechanism involves inducing inflammation. The effects of the selective JAK1-inhibitory anti-inflammatory drug upadacitinib on the side effects, histopathologic alterations, and kidney and in comparison, to silymarin and losartan, liver functions, oxidative stress, and inflammatory biomarkers were examined in male Wistar rats. In addition to receiving a single dosage of cisplatin (10 mg/kg) on the seventh day of treatment, the animals were given upadacitinib (10 mg/kg/day) for two weeks. Biochemical measurements were made of the oxidative biomarkers, inflammatory burst, liver, and kidney functioning, and more. Pretreatment with upadacitinib significantly improved liver function markers (ALT and AST) and prevented lipid profile abnormalities (triglycerides and total cholesterol) caused by cisplatin. Additionally, blood urea nitrogen, serum creatinine, creatinine clearance, and albumin levels all showed that it preserved kidney function. The lowering of MDA and TNFα levels suggests that upadacitinib also suppressed inflammatory processes produced by cisplatin in the liver and kidney. Furthermore, it enhanced the activity of superoxide dismutase (SOD). Upadacitinib significantly reduced the structural damage caused by histopathology in the liver and kidney tissues. Upadacitinib's reno protective effects were verified by western blotting of NF-kB and p-Akt. Additionally, the assay for cell viability demonstrates that upadacitinib had no inhibitory effect on the anticancer efficacy of cisplatin in MCF-7 and A549 cells. Furthermore, cisplatin's effectiveness against lung cancer cells has been enhanced by upadacitinib in a dose-dependent manner. These findings demonstrate how upadacitinib can prevent cisplatin-induced toxicity without compromising its anticancer properties.

Keywords: Upadacitinib, Cisplatin, Anticancer properties, ALT, AST

INTRODUCTION

Cisplatin is a platinum-based metalating agent used in chemotherapy that is frequently used as an

anticancer treatment. It is used to treat a variety of cancers, including tumors of the breast, lung, testicles, and ovaries. Notwithstanding its advantageous anticancer benefits, it has major side effects, including nephrotoxicity, neurotoxicity, ototoxicity, and hepatotoxicity at high dosages. The primary adverse effects that are dose-limiting among them are nephrotoxicity and hepatotoxicity, which develop gradually but unavoidably following the first and recurrent cisplatin administration. Additionally, several published studies in the field of clinical research have documented instances of cisplatin nephrotoxicity, as well as liver toxicity. Compared to other organs, the kidneys and liver accumulate more cisplatin, which at lower doses causes apoptosis and at greater levels necrosis. The process includes the release of pro-inflammatory cytokines, the infiltration of inflammatory cells into the kidneys, necrosis of the proximal tubule, apoptosis in the distal nephron because of inflammation and oxidative stress, and proximal tubular damage.

The cytotoxicity of cisplatin is ascribed to apoptosis, oxidative stress, and inflammation. DNA damage and cell injury are caused by declining antioxidant capability (e.g., by lowering the amounts of glutathione and protein-bound thiol groups). While cisplatin has been a vital chemotherapeutic agent, its application is restricted. After one to two weeks of treatment with a single dosage of cisplatin, 20–30% of patients experienced nephrotoxicity. Clearly, for safer clinical use, it is critical to identify strategies to mitigate the deleterious side effects of cisplatin at its cancer cell-killing dosages. Accordingly, in August 2019, the US Food and Drug Administration (FDA) approved upadaticinib, a Janus Kinase (JAK)-1 inhibitor, for the treatment of rheumatoid arthritis.

According to Serhal and Edwards (2019), upadaticinib functions by blocking proinflammatory cytokines such tumour necrosis factor (TNF) and interleukin-6 (IL-6), as well as JAK1, a crucial mediator of cell signalling. The FDA has lately warned about some side effects of upadacitinib, including blood clots, heart-related events, and cancer, despite the medication's therapeutic value. As part of its chemical mechanism of toxicity, cisplatin causes inflammation. We looked at upadacitinib's cytoprotective action against cisplatin-induced toxicities, particularly nephrotoxicity and hepatotoxicity, because of its anti-inflammatory properties. Protective agents that are effective can reduce toxicity while having no or a synergistic effect on their anticancer activity.

2. Materials and Methods

2.1. General

We bought upadacitinib from SVAK Life Sciences in pure powder form (Hyderabad, India). Cisplatin was utilised as a pharmaceutical solution (Cisplatine® Mylan (1 mg/mL), manufactured by Oncotec Pharma in Germany. We bought silymarin and losartan from a retail pharmacy. The remaining substances employed in this investigation were all of analytical grade. DiaSys Diagnostic Systems GmbH, Holzheim, Germany provided colorimetric assay kits for the following tests: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), total cholesterol (TC), creatinine, blood urea nitrogen (BUN), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), and commercial albumin kits.

CCr = [urine creatinine (mg / dl) × urine flow (ml / min)]/serum creatinine (mg / dl)

2.2. Experimental protocol

Five distinct groups, each including five rats, were created via random distribution of the rats.

Group I, the standard control group, was given 2-hydroxypropyl-β-cyclodextrin, p.o. for seven days, followed by a single intraperitoneal injection of isotonic saline on the eighth day. The vehicle was made by dissolving 2 g in 5 mL distilled water.

Group II consisted of rats that were administered with a single dosage of cisplatin (10 mg/kg, i.p.) after receiving a vehicle (2-hydroxypropyl-β-cyclodextrin, p.o.) for seven days (Koc et al., 2005, Pezeshki et al., 2017).

Group III was the cisplatin/losartan group (Cis/L); rats had cisplatin injection after receiving lossesartan (25 mg/kg/day in distilled water, p.o.) for a week. The next seven days saw the continuation of the losartan treatment.

Group IV consisted of rats that were injected with cisplatin at the end of the week after receiving 100 mg/kg/day of silymarin (100 mg/kg/day in 2-hydroxypropyl-β-cyclodextrin, p.o.). The next seven days saw the continuation of the silymarin treatment.

Group V consisted of rats that were injected with cisplatin on day seven after receiving upadacitinib (10 mg/kg/day in 2-hydroxypropyl-β-cyclodextrin, p.o.) for a week.

After that, the medication was administered for a further seven days.

Following a combined two-week period, rats were housed in metabolic cages without access to food or water for a full day. Urine samples were taken in order to calculate creatinine clearance (CCr), which is based on the amount of total protein and creatinine in the urine. The animals were put to sleep after the blood samples were taken, centrifuged for 15 minutes at 3000 rpm, and the serum was separated for the biochemical analyses. Each group was dissected, and the kidneys and liver were separated. The kidney/body weight ratio was calculated by removing the extra fat surrounding the kidneys and recording the weight. For histological investigations, a portion of the right kidney and liver were preserved in 10% formalin solution. For the Western blot examination, the right kidney's residual portion was utilised.The left kidney and a portion of the liver were promptly submerged in ice and homogenised in 0.1 M phosphate buffer (pH 7.4) at a ratio of 10% w/v. These samples were utilised to measure TNF-α levels and oxidative stress markers, SOD and MDA.

2.3. Assessment of nephrotoxic markers

Blood urea nitrogen (BUN), serum albumin, and serum creatinine were measured from the separated serum. Using the following formula, the CCr was used to calculate glomerular filtration rate based on serum and urine creatinine levels:

When the following formula was used to determine the urine flow per minute:

Urine flow (ml/min) = Urine volume in 24 h/ 1440

2.3.1. Calculating the urine's total protein

Pyrogallol red was used in photometric tests to determine total protein. Twenty microliters of purified water were pipetted into the blank test tube, and then twenty microliters of the sample was put to the sample test tube. Bovine serum was employed as a reference, and 1000 µL of reagent (sodium molybdate and pyrogallol red) was applied to each of the two test tubes. After mixing the

solution and letting it sit at 25°C for ten minutes, the absorbance at 600 nm was measured against the reagent blank.

2.3.2. Serum albumin measurement

The bromocresol green method was used for photometric assays to assess serum albumin levels. To prepare a blank, 10 µL of distilled water was pipetted into a test tube, and then 10 µL of the sample was added to the sample test tube. The two test tubes were filled with 1000 µL of reagent (bromocresol green and citrate buffer), using bovine serum as the standard. After mixing the solution and letting it sit at 25°C for 10 minutes, the absorbance was measured at 546 nm against the reagent blank.

2.3.4. Blood urea nitrogen measurement

Using the Urease GLDH: enzymatic UV test technique, serum urea was measured.

2.4. Hepatotoxic marker evaluation

Spectrophotometric techniques were used to measure the amounts of LDL-c and HDL-c, as well as cholesterol, triglycerides, ALT, and AST from the separated blood sera.

2.4.1. Serum cholesterol measurement

Using commercial diagnostic kits, the CHOD-PAP enzymatic photometric test method was used to measure serum cholesterol. A test tube was filled with 10 µL of distilled water, which served as a blank. The sample test tube was then filled with 10 µL of the sample. The two test tubes were filled with 1000 µL of the reagent, which included peroxidase, cholesterol esterase, 4-aminoantipyrine, phenol, and Good's buffer. After mixing the solution and letting it sit at 37°C for ten minutes, the absorbance was measured at 546 nm against the reagent blank.

2.4.2. Serum triglyceride measurement

Glycerol-3-phosphate-oxidase (GPO) was used in a colorimetric enzymatic test to measure serum triglycerides. In summary, 10 microliters of distilled water were pipetted into the blank test tube, and 10 microliters of the sample were put to the sample test tube. The two test tubes were filled with 1000 µL of the reagent (Good's buffer, 4-chlorophenol, ATP, Mg^{2+}, glycerol kinase, peroxidase, lipoprotein lipase, 4-aminoantipyrine, and glycerol-3-phosphateoxidase). After mixing the solution and letting it sit at 37°C for ten minutes, the absorbance at 546 nm was measured against the reagent blank.

2.4.3. Aspartate aminotransferases and alanine levels in the serum measured

following commercial diagnostic kits (DiaSys Diagnostic Systems) and an optimised UV test, serum ALT and AST were measured following the previously reported methodology.

2.4.4. Serum HDL-c and LDL-c measurements

Serum lipoprotein cholesterol was measured using an enzymatic reaction that produced colour. An enzymatic cholesterol test was used to determine the serum HDL-cholesterol level, as described.

2.5. Evaluation of liver and kidney tissue oxidative stress indicators

2.5.1. Malondialdehyde (MDA) level measurement

The competitive ELISA method was used to measure the lipid peroxidation. In this test, MDA-precoated microplates were utilised to compete with the MDA in the sample or standard for sites on the MDA-specific biotinylated detection Ab. The plate was cleaned of any excess conjugated

and unbound samples or standards. After adding horseradish peroxidase (HRP)-conjugated avidin to each well, the cultures were incubated. After that, each well received an addition of TMB substrate solution. The stop solution put an end to the reaction. A spectrophotometric approach was used to measure the colour change using a Robonik Readwell touch ELISA plate analyzer (Ambarnath, India) at a wavelength of 450 nm ± 2 nm. By comparing the optical density of the samples to the standard curve, the MDA concentration in the samples was determined.

2.5.2 Superoxide dismutase activity measurement

The antioxidative enzyme superoxide dismutase (SOD) catalyses the conversion of the superoxide anion into hydrogen peroxide and molecular oxygen. WST-1, which is reduced with a superoxide anion to yield a formazan dye, is used in the current colorimetric experiment. SOD inhibits the rate of reduction with a superoxide anion, which is linearly correlated with the activity of xanthine oxidase.

2.5.3. TNF-α level measurement

A pre-coated microplate containing a monoclonal antibody specific for rat TNF-α was prepared utilising the quantitative sandwich enzyme immunoassay. After adding standards, control, and samples to the wells, the immobilised antibody bound any TNF-α that was present. After that, washing was done to get rid of the loose materials. Subsequently, an enzyme-linked polyclonal antibody specific for rat TNF-α was added. Each well received a substrate solution after two hours of incubation and washing, and after thirty minutes of incubation, each well took on a blue hue. When the stop solution was added at the end, the mixture turned yellow in proportion to the quantity of TNF-α bound in the first step. After that, the sample values were calculated using the standard curve.

2.6 Analysing data

One-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparisons test were used for statistical analysis. The software GraphPad Prism was used to perform statistical analyses. It was considered that biochemical parameters had significance at $p < 0.05$.

3. Results

3.1. Upadacitinib and losartan's effects on nephrotoxicity indicators

Both the upadacitinib- and losartan-treated groups received a dosage of 10 mg/kg of cisplatin. In addition to other renal function indicators like BUN, serum creatinine, and serum albumin levels, the kidney-to-body weight ratio was evaluated.

When compared to the normal ($p < 0.01$), losartan ($p < 0.01$), and upadacitinib ($p < 0.05$) groups, the kidney to body weight ratio significantly increased in the cisplatin-treated group. Table 1 indicates that there was no statistically significant difference between upadacitinib treatment and lossartan treatment ($p > 0.05$ and $p > 0.05$, respectively).

Creatinine ($P < 0.001$), serum albumin ($P < 0.01$), urine protein ($P < 0.05$), and creatinine clearance ($P < 0.001$) in comparison to the normal control group. When upadacitinib was used in place of cisplatin, the raised level was much reduced. Table 1 shows that there was no discernible change in these indicators' levels between the normal, losartan, or upadacitinib groups.

Table 1 shows how cisplatin and upadacitinib affect indicators of nephrotoxicity.

	Normal Control	Cisplatin	Cisplatin/Losrtan	Cisplatin/Upadacitinib
Kidney body weight ratio × 10^{-3}	3.6± 0.11	7.05±0.76*	4.08±0.46[a]	4.30±0.20[a]
BUN (mg/dl)	18.2±1.4	80.7±7.8*	16.5±1.10[a]	22.6±2.3[a]
Serum creatinine	0.4±0.06	1.06±0.3*	0.25±0.05[a]	0.43±0.04[a]
Protein in urine	5.7±0.14	8.46±0.57*	6.16±0.16[a]	6.07±0.91[a]
Seum Albumin	2.68±0.15	3.48±0.14*	3.30±0.06[a]	3.08±0.19[a]

- *Significant difference from normal group.
- [a] Significant difference from cisplatin group
- The statistical mean ± standard error of mean (SEM) is used to present the data.

Table 2 shows how cisplatin and upadacitinib affect liver toxicity markers

	Normal	Cisplatin	Cisplatin/Silymarin	Cisplatin/Upadacitinib
Cholesterol	55.84± 3.9	7.05±0.76*	4.08±0.46[a]	4.30±0.20[a]
Triglycerides	28.02±2.11	80.7±7.8*	16.5±1.10[a]	22.6±2.3[a]
ALT	43.24±4.00	1.06±0.3*	0.25±0.05[a]	0.43±0.04[a]
AST	88.07±1.14	8.46±0.57*	6.16±0.16[a]	6.07±0.91[a]
LDL-C	20.98±1.05	3.48±0.14*	3.30±0.06[a]	3.08±0.19[a]
HDL-C	45.20±0.33	59.34±1.22*	38.71±4.77[a]	45.81±1.11[a]

- *Significant difference from normal group.
- [a] Significant difference from cisplatin group
- The statistical mean ± standard error of mean (SEM) is used to present the data.

3.2. Hepatotoxicity markers: the impact of silymarin and Upadacitinib

The liver indicators aspartate aminotransferase (AST), cholesterol, triglycerides, and serum alanine aminotransferase (ALT) were raised after receiving a 10 mg/kg injection of cisplatin in comparison to the normal group. Silymarin and upadacitinib treatment dramatically reduced the inflated indicators to levels that were almost normal. Table 2 demonstrates that there was no discernible change in these indicators' levels between the normal, silymarin, or upadacitinib groups.

As indicated in Table 2, further measurements of LDL-C and HDL-C were carried out to demonstrate the preventive effects of upadacitinib medication that counteract or lessen the negative effects of cisplatin.

3.3. Upadacitinib, losartan, and silymarin's effects on indicators of oxidative stress

The kidney and liver tissues of the cisplatin-treated group showed considerably higher levels of MDA ($P<0.001$) and TNF-α ($P<0.05$) than those of the normal group. When compared to rats

treated with cisplatin, the groups treated with upadacitinib and silymarin had a significant decrease in the elevated level of MDA-liver ($P<0.001$). Similarly, compared to the cisplatin group, the renal MDA level dropped in the upadacitinib and losartan groups ($P<0.01$). Rats administered upadacitinib had a considerably lower level of TNF-α in their liver tissue ($P<0.01$) as compared to rats treated with cisplatin. Between the medicine under investigation and the usual or silymarin-administered groups, there was no discernible difference. The improvement in the renal TNF-α level ($P>0.05$) was nearly attained with losartan.

When compared to the cisplatin control group, treatment with upadacitinib 10 mg/kg p.o. for 7 days markedly exacerbated the cisplatin-induced reduction in SOD activity in liver and kidney tissues ($P<0.05$ and $P<0.01$, respectively). The percentage of superoxide radical inhibition in liver tissue did not change significantly between the silymarin or upadacitinib groups ($P>0.05$), nor did the elevation of SOD activity in kidney tissues differ significantly between the normal, losartan, or upadacitinib groups ($P>0.05$).

3.4. Upadacitinib's impact on renal NF-kB and p-Akt expression levels produced by cisplatin

To verify upadacitinib's function in reducing cisplatin-induced toxicity, NF-kB expression in liver tissues was examined. In addition to the survival p-Akt's expression, which serves as a biological marker of the cell's reaction to apoptosis. The findings show that cisplatin treatment enhanced the expression of NF-κB p65 proteins in comparison to the control. In both (cisplatin + upadacitinib) and (cisplatin + losartan), the cisplatin-induced alterations were suppressed. In contrast to the cisplatin-treated group, p-Akt activation was restored in the (cisplatin + upadacitinib) and (cisplatin + losartan) groups.

Discussion

A platinum-based antineoplastic medication called cisplatin is used in chemotherapy to treat a variety of malignancies. Its extensive use has been hampered by major side effects such hepatotoxicity and nephrotoxicity. The literature reports served as the basis for determining the cisplatin dose and regimen used in this investigation. Furthermore, based on biochemical assessments of liver and kidney functions, we found that the 10 mg/kg dose is the most effective way to cause nephrotoxicity and hepatotoxicity in our initial pilot study, which included 5, 7, and 10 mg/kg doses. We determined the dosage of upadacitinib to be 10 mg/kg in accordance with a report in the literature. Transporters in the proximal tubule enable cisplatin absorption and excretion via the renal system. Injury, inflammation, and severe renal toxicity have a profound effect on renal proximal tubular cells once cisplatin builds up in them.

This has been demonstrated in the current study by the proximal tubules' damage, degeneration, and vacuolation caused by cisplatin. Additionally, it resulted in the infiltration of mononuclear cells, which demonstrated the presence of inflammation. Reduced glomerular filtration rate and elevated serum creatinine and BUN levels are indicative of tubular cell damage. The current study's findings on the atrophy of some renal corpuscles were another contributing element to this. It is unknown if these substances might reduce the antiproliferative effect of cisplatin, despite the fact that numerous candidates have been studied for their reno protective potential against cisplatin-induced nephrotoxicity. The goal of this investigation was to explore a unique combination of reno

protectives that either amplifies the anticancer effect of cisplatin with minimal toxicity or does not influence its efficacy.

According to our findings, cisplatin at a dose of 10 mg/kg decreased body weight and raised the kidney-to-body weight ratio when compared to normal. This could have been caused by injury to the renal tubules, which impacts water absorption and resulting in dehydration and a decrease in body weight.

The concentration of cisplatin in the kidney will be higher than that in the blood because of the buildup of the drug in renal parenchymal cells. Because of the failure of creatinine clearance, this destroys the renal cells and raises serum creatinine levels. This is consistent with our findings, which showed that the group treated with cisplatin had more serum creatinine than the control group. Urine with higher BUN, albumin, and protein levels is indicative of nephrotoxicity. When cisplatin was administered, this was seen in contrast to normal rats. These findings are consistent with previous reports and our histological results, which showed pathological alterations, support them. Renin-angiotensin-aldosterone pathophysiology is highly dependent on this system. In addition to numerous publications that employed losartan as a renoprotective medication in experimental and clinical studies, losartan, which blocks angiotensin II receptors, has been shown to protect against nephrotoxicity brought on by the use of cisplatin. This might be as a result of its capacity to inhibit JAK-1 selectively and to prevent the action of inflammatory Janus kinases. Brosius and He (2015) state that stimulation of the JAK-STAT pathway may have a role in the development of certain chronic renal disorders. The current study supported this by demonstrating that administering cisplatin in addition to upadacitinib reduced the kidney damage brought on by administering cisplatin alone. This later demonstrated upadacitinib's renoprotective properties.

Another hazard that limits the clinical usage of cisplatin is hepatotoxicity. According to Hoek and Pastorino (2002), cisplatin may cause harm and damage to liver cellular organelles by increasing the generation of proinflammatory cytokines and reactive oxygen species (ROS) while concurrently decreasing endogenous and exogenous antioxidants. The current investigation demonstrated damage to the hepatic cellular organelles by pyknosis, margination of other cells' nuclei, and eosinophilic degeneration of certain liver cells. There are two markers for hepatocellular damage: ALT and AST. Liver damage is associated with a considerable increase in these markers. The results of this study showed that the cisplatin group had higher ALT and AST levels than the normal control group, which suggests liver injury.

In a similar vein, our investigation revealed a statistically significant increase in triglyceride and cholesterol levels in cisplatin-treated rats compared to control rats. This could be explained by the generation of ROS, which eventually lead to peroxidation and protein denaturation, which harm the lipid components of the cell membrane.

Silymarin, an antioxidant flavonoid complex derived from the herb milk thistle, was able to reverse hepatic cell injury. It also stabilises membrane permeability, inhibiting lipid peroxidation and reducing or preventing glutathione depletion caused by hepatotoxicity. Furthermore, silymarin has a prophylactic effect on rats by preventing hepatic histopathological changes caused by cisplatin. It revealed that the liver tissue resembled the control rat liver in appearance. Numerous studies

have documented the important function that the PI3K/Akt pathway plays in protecting the kidneys by stimulating cell differentiation, inhibiting the pro-apoptotic protein BAD, and activating anti-apoptotic proteins including Bcl-2 and Bcl-xl. Phosphorylated Akt expression was restored in the upadacitinib-treated group relative to the cisplatin-treated group in our investigation, which is in line with these published data. Furthermore, pro-inflammatory mediators like TNF-α, IL-1, IL-6, IL-18, iNOS, and COX-2 are regulated by the NF-κB pathway, which ultimately plays a role in acute renal damage. Based on our findings, upadacitinib and losartan both have kidney protective effects against cisplatin-induced nephrotoxicity via inhibiting NF-κB expression and having anti-inflammatory properties. Notably, we did not see the formation of blood clots when using the upadacitinib dosage regimen that was examined.

Additionally, testing whether upadacitinib anti-inflammatory and protective properties lessened cisplatin's anticancer effects showed that it had no influence on the cytotoxicity of cisplatin in MCF-7 and A549 cells. It's interesting to note that upadacitinib has, in a dose-dependent way, somewhat increased the potency of cisplatin in A549 cells, a lung cancer model. Since several JAK inhibitors are currently exhibiting encouraging results in clinical trials as anticancer medications, more thorough research into the antitumor activity of upadacitinib alone or in combination with cisplatin should be conducted to explore the precise mechanism of action.

Conclusion

Nephrotoxicity and hepatotoxicity in particular, which are caused by the chemical cisplatin, have been two of the main obstacles to cancer treatment. Based on the outcomes of Western blot and histological investigations along with kidney and liver biomarkers, this study has demonstrated the advantageous impacts of upadacitinib in addressing cisplatin-induced hepatotoxicity and renal toxicity without sacrificing cisplatin's anticancer efficacy. It is advised to carry out a lengthy investigation to examine the potential therapeutic benefits of various upadacitinib dosages, as well as the preventative benefits and potential induction of side effects upon testing greater doses of the medication.

In tests using A549 lung cancer cells, upadacitinib and cisplatin together not only did not lessen the death impact of cisplatin, but also enhanced it. This synergistic effect occurred in a dose-dependent manner. A further study to investigate this observation at molecular level is planned to be carried out in the future, and the results will be published in the due course.

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