

# Altered cannabinoid receptor expression in pancreatic islets in experimental model of uraemia

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[Received: 8 July 2019; Accepted: 21 September 2019]

**Background:** Uraemia leads to a number of metabolic and hormonal disorders including defective carbohydrate metabolism. Endocannabinoids exert their effect on insulin and glucagon secretion via activation of specific receptors named CB1 and CB2. For this reason and the absence of reports on location and immunoreactivity of CB1, CB2 receptors compared to immunoreactivity of insulin- and glucagon-secreting cells in experimental uraemia, the author decided to investigate this issue. The aim of the present study was the immunohistochemical localisation and evaluation of cannabinoid receptors (CB1, CB2), insulin and glucagon in the pancreatic islets of uraemic rats.

**Materials and methods:** Fragments of the rat's pancreas were collected 28 days after surgical resection of one kidney and removal of 70% of the other kidney cortex. Paraffin-embedded sections were stained with haematoxylin-eosin and immunohistochemical reactions were performed with the use of a specific antibody against CB1-, CB2-receptors, insulin and glucagon.

**Results:** It was revealed the decreased immunoreactivity of the CB1 receptor and higher intensity of the immunohistochemical reaction against CB2 receptor as compared to the value in the control animals. Significantly higher immunoreactivity of glucagon-positive cells and weaker immunoreactivity of insulin-positive cells were observed in pancreatic islets of uraemic rats.

**Conclusions:** The obtained results indicate the involvement of cannabinoid receptors in the pathomechanism of carbohydrate metabolism disorders, associated with abnormal secretion of hormones by the  $\alpha$  and  $\beta$  cells in uraemia. (Folia Morphol 2020; 79, 3: 469–475)

**Key words:** pancreatic islets, cannabinoid receptor, uraemia, rat

## INTRODUCTION

It is well known that chronic renal failure results in disorders of many organs and systems including carbohydrate metabolism [2, 3, 15]. Recent studies indicate a close functional relationship between the endocannabinoid system and hormones related to maintaining the energy and metabolic balance [18, 23, 30, 33].

Due to the presence of CB1 and CB2 receptors in pancreatic islet cells, endocannabinoids are involved in the regulation of insulin and glucagon [1, 5, 6, 22, 35]. Several reports indicate that using CB1 receptor antagonist results in impaired insulin secretion and administration of an agonist leads to increased insulin secretion in rats [11, 13, 22]. Similar observations

have been made on patients treated with a CB1 receptor agonist [7]. Other studies on mice have shown a decrease in insulin secretion following CB1 receptor activation [28].

It has been proven in experimental conditions that following the use of streptozotocin,  $\beta$  cell survival increases in animals with a pharmacological blockade or a genetic deficiency in the CB1 receptor. The same experiment revealed that the activation of this receptor induces the death of  $\beta$  cells in an insulin receptor-dependent manner [19].

Recent reports indicate the presence of autonomous endocannabinoid system in the endocrine pancreas and emphasize important role of CB1 receptors in  $\beta$  cells. In addition, the participation of  $\alpha$  pancreatic islet cells in the synthesis of endocannabinoids and  $\beta$  cells in the synthesis of enzymes degrading endocannabinoids has been demonstrated [7, 25, 26]. Activation of CB1 receptors by cannabinoids produced in  $\alpha$  cells may lead to an increase in insulin secretion in  $\beta$  cells [25].

Endocannabinoids are synthesized on demand from derivatives of arachidonic acid bounded to membrane. After action, the endocannabinoids are rapidly degraded by specific enzymes and products are recycled. CB1 and CB2 are G-protein coupled receptors and inhibit activation of adenylyl cyclase (AC) and cAMP-PKA activity as well as activate MAPK. In addition, CB1 receptor inhibits voltage-gated L-, N- and P/Q-type  $\text{Ca}^{2+}$  channels and inwardly correcting  $\text{K}^+$  channels [14].

There are many pathways activated by the CB1 and CB2 receptors associated with the secretion and survival of pancreatic islet cells. CB1 receptor may induce activation of an inwardly rectifying  $\text{K}^+$  channel, resulting in decrease excitability and inhibition of voltage gated calcium channel and inhibition of  $\text{Ca}^{2+}$  influx. Activated CB1 or CB2 receptor leads to the activation of p38 and p42/44 mitogen activated protein kinases (MAPKs). The P38 isoforms of MAPK can activate caspases and induce apoptosis, on the other hand p42/p44 stimulates cellular proliferation. Gi/o activation evoked by CB1 and CB2 receptors indicates AC inhibition and subsequent reductions in cyclic AMP (cAMP). Under certain circumstances CB1 receptors may increase  $\text{Ca}^{2+}$  influx induced by stimulation AC via Gs and the increased cAMP, then it can activate protein kinase A and result in phosphorylation of VGCCs. Calcium level may be elevated also by activation of phospholipase C via another pathway related with CB1 receptor. Entry into this

pathway may also result in stimulation of cellular proliferation [23].

It is well known that renal failure leads to the accumulation of many toxic substances such as urea or reactive oxygen species which can impair the function and impact on the survival of a number of cell types inducing their apoptosis [20, 29, 34].

In the current literature there is a lack of reports concerning cannabinoid receptors in the pancreatic islet area in uraemia.

The aim of the study was the immunohistochemical localisation of CB1, CB2 cannabinoid receptors and insulin-, glucagon-secreting cells in the pancreas of uraemic rats.

## MATERIALS AND METHODS

### Experimental animals

The study was performed on 15 young male Wistar rats, their body weight at the beginning of the experiment within 200–220 g (the mean body weight:  $210 \pm 10$  g). The animals were kept in lighted and ventilated conditions with room temperature and maintained day and night rhythm. The animals had a free access to standard granulated chow and drinking water was available. All the experiments were performed at the same time of the day. Procedures involving the animals and their care were conducted in conformity with the institutional guidelines that were in compliance with national and international law and with guidelines for the use of animals in biomedical research. The experimental rats were divided into two groups: SH — five animals underwent a sham operation and experimental uraemic group, U — ten rats with experimentally induced uraemia according to the method described by Ormrod and Miller: surgical resection of one kidney and removal of 70% of the other kidney cortex.

### Method of experimental material collection and fixation

After 4 weeks from the surgery, the rats were anaesthetised by pentobarbital, administered intraperitoneally (i.p.) at a dose of 50 mg/kg and blood was collected from their hearts. Then, the animals were sacrificed by decapitation. The pancreas was immediately removed. For microscopic analysis segments of the pancreatic distal parts were used, fixed in Bouin's fluid and embedded in paraffin in the routine way. The specimens were cut into 4  $\mu\text{m}$  slices (Leica 2025 Autocut) and stained by haematoxylin and eosin (H+E) for general histological examination.

### Determination of urea and creatinine levels in blood serum

Blood was collected from the heart for coagulation. Collected blood samples were left for 20 min in room temperature to coagulate. Then, the cylinders with coagulated blood were centrifuged at 3000 RPM for 15 min. In the blood serum obtained, urea and creatinine levels were measured in a Backman-CX4 Analyser, using an "Urée cinétique UV 800" of BioMérieux.

### Immunohistochemical procedure

Immunohistochemical reactions against CB1, CB2, insulin, glucagon were performed on 4 µm paraffin sections, obtained from the pancreas of the studied animals.

Briefly: Sections were deparaffinised and hydrated with pure ethanol. In the immunohistochemical study, the EnVision method was used according to Herman and Elfont [16]. Antigen retrieval is recommended before commencing IHC staining: for glucagon is Target Retrieval Solution (S1700; Dako, Denmark), and for CB-1 and CB-2 is Target Retrieval Solution pH = 9.0 (S2367; Dako, Denmark). Tissues were blocked in Peroxidase Blocking Reagent (S2001 Dako Denmark A/S, Produktionsvej 42, DK-2600 Glostrup) for 10 min at room temperature. Sections were incubated in humidified chamber with dilution primary antibodies: insulin 1:100 (A0564 Dako) and glucagon 1:200 (A0565 Dako) (30 min RT); CB-1 (ab23703 ABCAM) 1:200 and CB-2 (ab3561 ABCAM) 1:2000 (24 h in +4°C). HRP polymer KIT (EnVision(+)) HRP polymer anti-rabbit K 4011 Dako Denmark A/S, Produktionsvej 42, DK-2600 Glostrup) was used as the secondary antibody followed by colorimetric detection using chromogen DAB. Sections were counterstained with haematoxylin QS (Vector) and dehydrated with pure ethanol and xylene to prepare for mounting.

### Quantitative analysis

The analysis of the preparations and their photographic documentation were performed using an Olympus BX41 microscope with a digital camera (Olympus DP12) and standard morphometric programme (NIS-Elements Advanced Research software of Nikon) installed on a computer. Ten randomly selected islets in each section were chosen, at a magnification 200× (20× the lens and 10× the eyepiece) for further morphometric analysis. The intensity of immunohistochemical reactions for each antibody was analysed. Intensity of immunohistochemical reaction was meas-

**Table 1.** Serum concentrations of creatinine and urea in control and uraemic rats

	Control	Uraemic	P
Creatinine [mg/dL]	0.52 ± 0.052	0.72 ± 0.12	< 0.05
Urea [mg/dL]	35.33 ± 5.98	85.58 ± 9.766	< 0.05

ured by using 0 to 256 grey scale levels, where a completely black pixel got a value of 0, whereas one with a value of 256 is completely white or bright.

### Ethical issues

Study assumptions, aim, schedule and mode of animal treatment were approved by the Senate Committee for Supervision of Experiments on Humans and Animals, Medical University of Białystok.

### Statistical analysis

The analysis was performed using the Statistica Version 10.0 programme. Results are expressed as means ± standard deviation. The corresponding mean values were computed automatically; significant differences were determined by Student's t-test;  $p < 0.05$  was taken as the level of significance.

## RESULTS

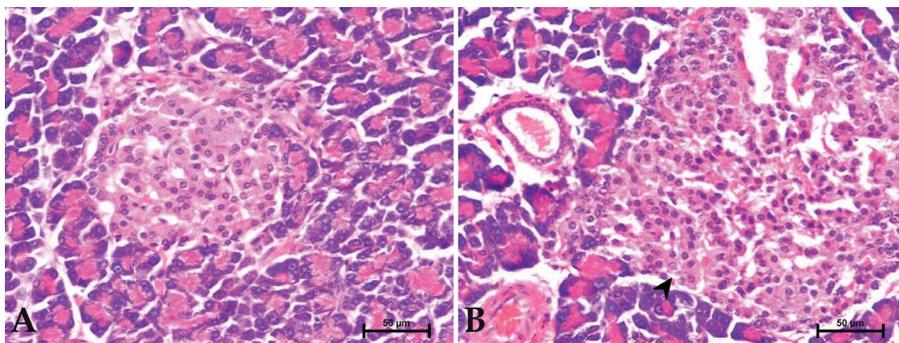
Serum creatinine and urea levels in rats subjected to unilateral nephrectomy and partial decortication of the other kidney were significantly increased in experimental animals in comparison to the values of those parameters in control rats (Table 1).

Routine H+E staining tests have revealed differences in islet morphology between both groups. In uraemic rats, the pancreatic islets have a larger surface area and a more irregular shape compared to rats with normal renal function (Fig. 1A, B).

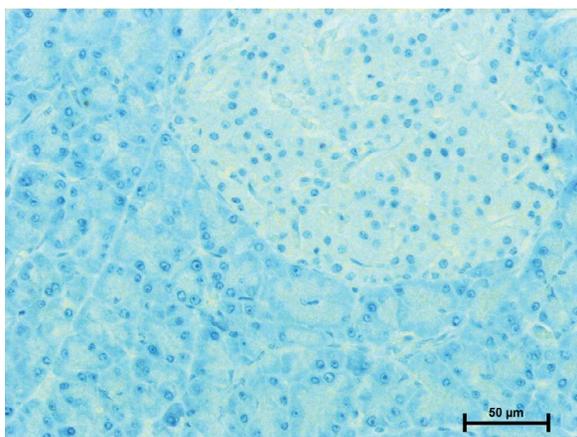
A representative image of CB1-negative control (Fig. 2). All negative controls were similar. In each case omission of the primary antibody in immunohistochemical staining resulted in a lack of reaction.

A positive reaction to CB1 and CB2 receptors was observed in the majority of pancreatic islet cells of all tested rats. The intensity of immunoreaction and distribution of these receptors were different in particular groups of animals.

In control rats, strong or very strong immunoreactivity against CB1 receptors was observed throughout the islets of Langerhans. In the pancreas of rats with renal insufficiency, the intensity of the CB1-positive



**Figure 1.** Photomicrographs of pancreatic islet of control rat (A), uraemic rat (arrow head) (B) (original magnification  $\times 200$ ); haematoxylin and eosin stain.

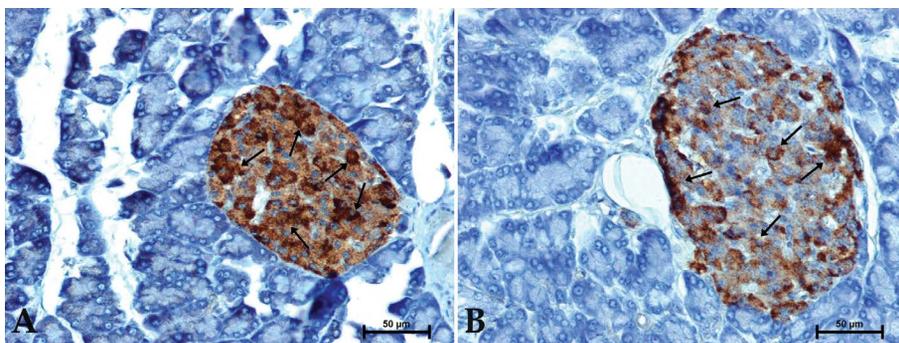


**Figure 2.** Representative image of negative control reaction. Lack of reaction after omission of the primary antibody in immunohistochemical procedure (original magnification  $\times 200$ ).

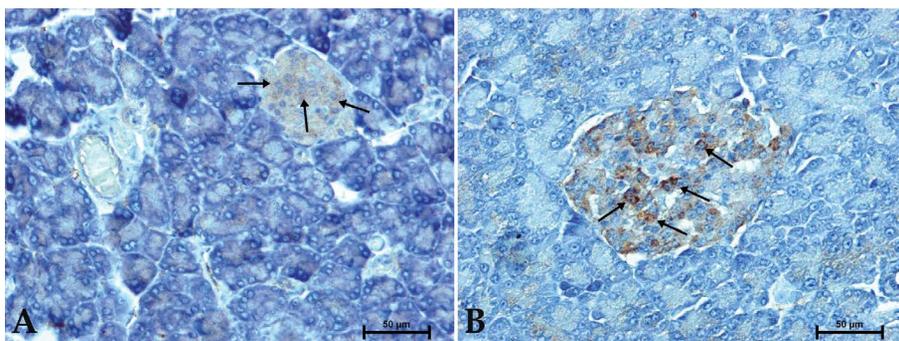
reaction was significantly weaker compared to the control animals and the density of CB1 receptors was higher on islets circumference (Fig. 3A, B).

The use of an anti-CB2 antibody resulted in a very weak or marginal reaction in the pancreas of control rats (Fig. 4A). The result of the CB2-positive reaction was significantly greater in the pancreas of uraemic rats. Some cells in the central parts of uraemic rats' pancreatic islets showed moderate or strong immunoreactivity against CB2 (Fig. 4B).

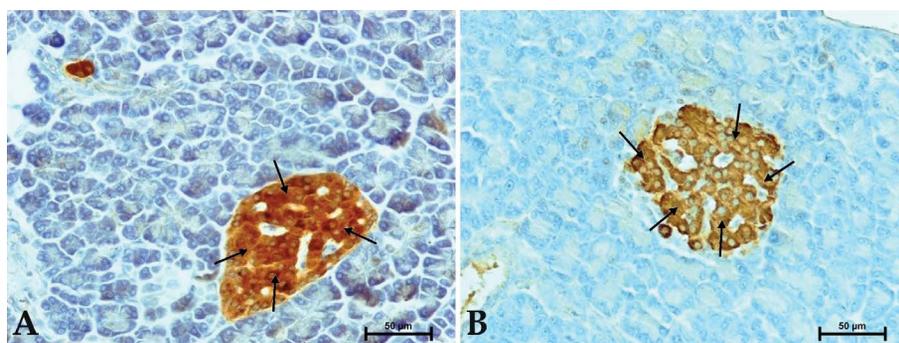
Immunohistochemical tests showed a typical arrangement of insulin- and glucagon-producing cells in all the examined rats. Insulin-positive cells were observed over the entire surface of the pancreatic islets (Fig. 5A, B), while glucagon-containing cells occurred primarily on islets periphery (Fig. 6A, B).



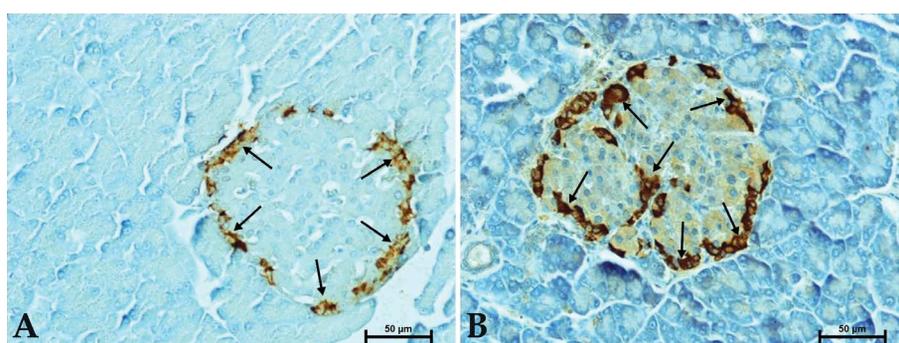
**Figure 3.** Immunodetection of CB1 receptor in pancreatic islet of control rat (A), uraemic rat (arrows) (B) (original magnification  $\times 200$ ).



**Figure 4.** Immunodetection of CB2 receptor in pancreatic islet of control rat (A), uraemic rat (arrows) (B) (original magnification  $\times 200$ ).



**Figure 5.** Immunodetection of insulin in pancreatic islet of control rat (A), uraemic rat (arrows) (B) (original magnification  $\times 200$ ).



**Figure 6.** Immunodetection of glucagon in pancreatic islets of control rat (A), uraemic rat (arrows) (B) (original magnification  $\times 200$ ).

**Table 2.** Comparative analysis of intensity of immunohistochemical reaction for CB1, CB2, insulin, glucagon in pancreatic islets of control and uraemic rats

Group	Intensity of immunohistochemical reaction in scale from 0 (black pixel) to 256 (white pixel)			
	CB1	CB2	Insulin	Glucagon
Control	53.9 $\pm$ 2.98	175.5 $\pm$ 1.46	86.3 $\pm$ 3.7	115.1 $\pm$ 1.57
Uraemia	104.2 $\pm$ 3.67*	116.7 $\pm$ 3.40*	116.1 $\pm$ 7.07*	75.2 $\pm$ 5.02*

Data are shown as the mean value  $\pm$  standard deviation; \* $p < 0.05$  uraemia vs. control

The intensity of the immunohistochemical reaction revealing insulin in uraemic rats was attenuated compared to the control group (Fig. 5).

The intensity of the glucagon revealing immunoreaction in pancreatic islet  $\alpha$  cells of the control group was moderate. Significantly higher immunoreactivity and an increased number of glucagon-positive cells were observed in pancreatic islets of uraemic rats (Fig. 6A, B).

Computer analysis confirmed visually observed changes in reaction intensity (Table 2).

## DISCUSSION

The relationship between insulin-secreting  $\beta$  cells and glucagon-secreting  $\alpha$  cells, which work together to maintain glucose homeostasis, within the pancreatic islets is an area of extensive research. A novel component of energy regulation, endocannabinoids,

has recently been identified within the endocrine part of the pancreas.

Homeostatic disorders occurring in uraemia, within a short period of time manifest themselves in the abnormal secretion of hormones and other biologically active substances. It is known that the endocannabinoid system is involved in the regulation of the energy management of the body since elements of this system occur in key peripheral tissues that control metabolism, i.e. liver, adipose tissue, muscles, and in the endocrine part of the pancreas [2, 23, 33]. Cannabinoid receptors CB1, CB2 are present in pancreatic islet cells and are involved in the regulation of hormones secreted by them [13, 22, 30].

The aim of this study was to investigate, for the first time, the distribution of CB1 and CB2 cannab-

inoid receptors as well as insulin- and glucagon-secreting cells in the pancreas of uraemic rats.

The results obtained in the experiment indicate statistically significant differences in the examined parameters in uraemic and control rats.

In this experiment, the CB1 and CB2 receptors were found to be present in the rat pancreatic islets as well as the immunoreactivity of the CB2 receptor was significantly lower than that of CB1. Likewise, other experimental studies conducted by Bermúdez-Silva et al. [6] also have demonstrated presence of CB1 and CB2 receptor immunoreactivity in endocrine pancreas of male Wistar rats.

Our study demonstrated diminished immunoreactivity of the CB1 receptor in uraemic rats in comparison to controls. The CB1 receptor density was higher at the periphery of the pancreatic islets in the examined rats whereas the intensity of the immunohistochemical reaction against the CB2 receptor was significantly higher in single pancreatic islet cells in uraemic rats. Considering that in the current literature there is lack of reports concerning evaluation of cannabinoid receptors in pancreas in uraemia, the discussion is quite difficult.

Several studies show that the effects of activation or blockade, as well as the distribution of cannabinoid receptors in humans and in rats, are very similar [5, 6, 7, 11, 22]. Laychock et al. [22] showed an increase in insulin secretion in isolated rat pancreatic islets after the administration of  $\Delta$ -9-tetrahydrocannabinol [22]. Bermúdez-Silva et al. [7] observed an increase in insulin as well as glucagon secretion in human pancreatic islets after the administration of a CB1 receptor agonist. In contrast, CB2 receptor stimulation reduced insulin secretion [7]. Getty-Kaushik et al. [13] found decreased insulin secretion in pancreatic islets of obese Zucker and Zucker Diabetic Fatty rats treated with a CB1 receptor antagonist.

The decrease in the CB1 receptor immunoreactivity observed in the present study in the central part of the islets may indicate the induction of protective mechanisms in the pancreatic islets in chronic renal failure. Confirmation of this hypothesis may be found in studies conducted by Janiak et al. [17] as well as Kim et al. [19]. Janiak et al. [17] used a CB1 receptor antagonist and observed the preservation of pancreatic weight and  $\beta$  cell mass in obese Zucker rats. Kim et al. [19] found that the blockade or genetic deficiency in the CB1 receptor increases the survival of  $\beta$  cells in mice following the administration

of streptozotocin. Other studies conducted by Lin et al. [24] demonstrated that blockade of CB1R by treatment with CB1R antagonist attenuates the left ventricular hypertrophy and Akt-mediated cardiac fibrosis in chronic kidney disease mouse model. On the other hand, Bátkai et al. [4] showed protective role of CB2 receptor activation in hepatic ischaemia/reperfusion injury in mice. Similar observations were made by Montecucco et al. [27] in hearts from a mouse model of ischaemia/reperfusion. It is known that chronic kidney disease leads to increase in number of different substances provoking cell damage and apoptosis therefore observed decrease in CB1 receptor and increase in CB2 immunoreactivity may be one of the possible adaptation processes aimed at limiting pancreatic islet cell destruction.

In the pancreas of uraemic rats, lower intensity of the insulin-positive reaction as well as stronger immunoreactivity of glucagon-positive cells were observed. Hyperglucagonaemia in chronic renal failure is a phenomenon that has been repeatedly mentioned in various reports [8, 9, 12, 31]. The results of studies conducted by Koppe et al. [20] show defective insulin secretion caused by the direct action of urea on  $\beta$  cells. On the other hand, insulin resistance which develops during chronic renal failure also has a profound effect on the function of endocrine cells in the pancreatic islets [10, 21, 32, 36].

Several reports published to date illustrate that CB1 receptor antagonists have an inhibitory effect on insulin secretion [7, 10, 13]. Considering the information presented above and the results of our own research, it can be assumed that there may be CB1 blocking agents in chronic renal failure. However, changes observed in our study may be the result of a number of different mechanisms and it is difficult to explain them at this stage since the body of knowledge regarding this issue is limited.

## CONCLUSIONS

It can be concluded that uraemia leads to disorders of the insulin, glucagon production and CB1, CB2 immunoreactivity in the pancreatic islets as well as larger surface area and a more irregular shape of pancreatic islets in uraemic rats compared to rats with normal renal function. That suggests the involvement of cannabinoid receptors in the pathomechanism of carbohydrate metabolism disorders in chronic kidney disease.

The results of our studies can contribute to a better understanding of the changes occurring in

the endocrine part of the pancreas in uraemia as well as inspire other scientists to conduct research involving this important issue.

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