Al-Rafidain J Med Sci. 2024;6(1):172-178.
DOI: https://doi.org/10.54133/ajms.v6i1.566

Research Article

Expression of BAX and eNOS in Rabbit Pancreatic Tissues Injured by Hydrocortisone

Ahmed Fakhir Hameed[1], Khalida Ibrahim Noel[1]*, Mohammed Emad Shukri[2], Khalid Muhsin Hassan[1]

[1]*Department of Human Anatomy, College of Medicine, Mustansiriyah University, Baghdad, Iraq;* [2]*Department of Anatomy, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq;* [3]*Baghdad Teaching Hospital, Medical City, Baghdad, Iraq*

Received: 12 January 2024; Revised: 14 February 2024; Accepted: 18 February 2024

Abstract

Background: There have been cases of acute pancreatitis brought on by steroids, but identifying it is challenging and necessitates careful monitoring. However, according to new research, 3–5% of all cases of illness may be caused by drug-induced acute pancreatitis, making it the third most common cause. ***Objective***: Evaluation of the effect of hydrocortisone injections on pancreatic structure immunohistochemically using BAX and eNOS markers. ***Methods***: White New Zealand female rabbits weighing between 1.2 and 1.5 kg were used, and they were given free access to food. The rabbits were split into six groups, with five animals in each group receiving intramuscular hydrocortisone injections for 14 and 21 days, respectively, at a dose of 5 mg and 20 mg/kg for short and long durations, and two control groups. ***Results***: There was an increase in weight in both long-duration groups (GL1 and GL2) after week 2 of injection when compared to both control and short-duration groups. There was a highly statistical difference in the expression of BAX in both short- and long-duration groups compared to the control group, and there was also a decrease in the expression of BAX when duration increased. Similarly, there was a highly statistical difference in the expression of eNOS in both the GS and GL groups when compared to the control group. ***Conclusions***: The pancreas can be injured by high and low doses of hydrocortisone if used for more than 2 weeks.

Keywords: BAX, eNOS, Hydrocortisone, Immunohistochemistry, Pancreas.

التعبير عن BAX و eNOS في أنسجة بنكرياس الأرنب المتضررة نتيجة التعرض للهيدروكورتيزون

الخلاصة

الخلفية: هناك حالات من التهاب البنكرياس الحاد الناجم عن الستيرويدات، ولكن تحديده يمثل تحديا ويتطلب مراقبة دقيقة. ومع ذلك، قد يكون سبب 3-5% من جميع حالات التهاب البنكرياس الحاد الناجم عن الأدوية، مما يجعله السبب الثالث الأكثر شيوعا. **الهدف**: تقييم تأثير حقن الهيدروكورتيزون على بنية البنكرياس المناعية الكيميائية باستخدام علامات BAX وeNOS . **الطريقة**: تم استخدام إناث الأرانب النيوزيلندية البيضاء التي يتراوح وزنها بين 1.2 و 1.5 كجم، وتم منحها حرية الوصول إلى الطعام. تم تقسيم الأرانب إلى ست مجموعات، حيث تلقت خمسة في كل مجموعة حقن الهيدروكورتيزون العضلي لمدة 14 و 21 يوما على التوالي، بجرعة 5 ملغ و 20 ملغم/كغم لفترات قصيرة وطويلة، ومجموعتين ضابطتين. **النتائج**: كانت هناك زيادة في الوزن في كلتا المجموعتين طويلتي الأمد (GL1 و GL2) بعد الأسبوع الثاني من الحقن مقارنة بكل من المجموعة الضابطة وقصيرة المدة. كان هناك اختلاف إحصائي كبير في التعبير عن BAX في كل من المجموعات قصيرة وطويلة الأجل مقارنة بالمجموعة الضابطة، وكان هناك أيضا انخفاض في التعبير عن BAX عند زيادة المدة. وبالمثل، كان هناك فرق إحصائي كبير في التعبير عن eNOS في كل من المجموعتين GS و GL عند مقارنتها بالمجموعة الضابطة. **الاستنتاجات**: يمكن أن يصاب البنكرياس بجرعات عالية ومنخفضة من الهيدروكورتيزون إذا تم استخدامه لأكثر من أسبوعين.

* ***Corresponding author***: Khalida I. Noel, Department of Human Anatomy, College of Medicine, Mustansiriyah University, Baghdad, Iraq; Email: dr.khalidanoel@uomustansiriyah.edu.iq

Article citation: Hameed AF, Noel KI, Shukri ME, Hassan KM. Expression of BAX and eNOS in Rabbit Pancreatic Tissues Injured by Hydrocortisone. *Al-Rafidain J Med Sci*. 2024;6(1):172-178. doi: https://doi.org/10.54133/ajms.v6i1.566

© 2024 The Author(s). Published by Al-Rafidain University College. This is an open access journal issued under the CC BY-NC-SA 4.0 license (https://creativecommons.org/licenses/by-nc-sa/4.0/).

INTRODUCTION

Glucocorticoids can be defined as wide-spectrum inflammation-suppressing medications that have a wide-ranging anti-inflammatory effect [1]. Glucocorticoids are multifaceted and can have several adverse effects, such as elevated blood sugar, secondary infections, weakened bones, wound healing dilemmas, and gastrointestinal bleeding [2].

Currently, asthma, chronic obstructive pulmonary disease (COPD), and systemic vasculitis are treated with glucocorticoids, and their effects are unquestioned [3]. They are also considered to have advantageous, yet controversial, effects on septic shock, community-acquired pneumonia, burns, and acute respiratory distress syndrome (ARDS) [4]. Recently, it has been reported that patients with COVID-19 may also benefit from the use of glucocorticoids to counter inflammatory storms [5]. Drugs were previously believed to be a very uncommon cause of acute pancreatitis, with incidence rates ranging from 0.1 percent to 2 percent [6]. The condition is frequently mild to moderate in severity and does not usually result in complications. There have been cases of steroid-induced acute pancreatitis, although diagnosing it is difficult and necessitates close observation [7]. But according to recent estimates, 3–5% of all occurrences of the condition may be initiated by drug-induced acute pancreatitis, making it the third most common cause [8]. Approximately 1% of the population uses oral glucocorticoids, one of the most popular drug classes [9]. In addition to peptic ulcers, diabetes mellitus, and osteoporosis [10], Acute pancreatitis induction has been reported as a significant adverse effect in multiple case studies [11]. Pancreatitis is an important clinical problem presented as an acute abdomen in clinical practice with a high death rate, between 15 and 40 percent, for which treatment remains largely supportive [12]. Corticosteroids have been shown to be beneficial in the treatment of acute pancreatitis [13]. As an immediate inflammatory response to pancreatic complaints, acute pancreatitis necessitates critical alterations in the expression of several pancreatic genes [14]. It is a serious condition that develops quickly, lasts a long time, and progresses quickly. It causes severe necrosis of the pancreatic tissue, multiple organ failures outside the pancreas, and a high death rate. Over the past few decades, the mortality rate from subacute pancreatitis (SAP) has gradually decreased despite advancements in treatment methods, and the exact pathophysiology of SAP remains unclear [15]. But as possible treatment targets, specialists have concentrated on inflammatory mediators and their related antagonists, which often leads to patients dying from the illness [16]. The pathophysiology of subacute pancreatitis (SAP) involves apoptosis, the production of inflammatory mediators, disruption of the microcirculation, and pancreatic activation. According to recent research, apoptosis may be a helpful response. Apoptotic acinar cells can both cause damage and reduce inflammation [17]. Several practical studies have demonstrated that glucocorticoids can increase the survival rate of animals with acute pancreatitis [18], either by blocking the production of inflammatory mediators alone or in combination with their inhibition of effects, reducing overall body stress, improving microcirculation, reducing endotoxemia, eliminating free radicals, blocking nitric oxide (NO) and NF-κB expression, etc. Research has shown that the therapeutic benefit of large doses of dexamethasone administered early on is much greater than that of tiny doses [19]. Numerous studies have demonstrated the effectiveness of corticosteroids in the management of acute pancreatitis [20]. Research has demonstrated that the group receiving dexamethasone treatment had a higher apoptotic index along with less severe inflammation, bleeding, and pancreatic necrosis. This suggests that dexamethasone can either directly induce pancreatic cell apoptosis or indirectly induce apoptosis by preventing an excessive increase in TNF-α, IL-6, PLA2, and other related chemicals [21]. Humans with the BAX gene generate the protein BAX, commonly referred to as bcl-2-like protein 4. BAX is a member of the Bcl-2 gene family. Both Bcl-2-associated X protein (BAX) and Bcl-2 homologous antagonist/killer (BAK) are regarded as apoptosis controllers. In vivo, both molecules interact with one another and send out signals that control IRE1/XBP1 signaling [22]. To improve the presence of injured pancreatic tissue, eNOS has been tested in this study. Constitutive NOS, also known as nitric oxide synthase 3 (NOS3) (eNOS), commonly referred to as endothelial NOS (eNOS), is an enzyme that the human NOS3 gene encodes [23]. However, eNOS, which generates NO at nanomolar levels, also contributes to inflammation. For instance, the proinflammatory molecules nuclear factor-B (NF-B) and cyclooxygenase-2 can be expressed under the control of eNOS [24]. Hydrocortisone is a steroid hormone; it has both glucocorticoid and mineralocorticoid action. It is widely used in many diseases as an anti-inflammatory drug [25], and it is also regarded as an agonist of the endogenous glucocorticoid and mineralocorticoid. It also has an immune suppression effect, and due to its anti-allergic effect, it is successfully prescribed for anaphylaxis shock. Hydrocortisone also substitutes for the endogenous corticosteroid in cases of adrenal crisis [26]. This study aimed to compare the different doses and durations of hydrocortisone administration on the pancreas of rabbits immunohistochemically.

METHODS

Study Design

Healthy female white New Zealand rabbits weighing about 1.2–1.5 kg were used, fed *ad libitum.* The female rabbits were divided into six groups, five animals in each, as follows: GS1 (group short duration 1): treated by intramuscular injection of 5 mg/kg of hydrocortisone sodium succinate (SOLU-CORTEF® USA) for 14 days in the thigh muscle. GS2 (group short duration 2) was treated by intramuscular injection of 20 mg/kg of hydrocortisone sodium succinate for 14 days in the thigh muscle. GL1 (group long duration 1) was treated by intramuscular injection of 5 mg/kg of hydrocortisone sodium succinate for 21 days in the thigh muscle. GL2 (group long duration 2) was treated by intramuscular injection of 20 mg/kg of hydrocortisone sodium succinate for 21 days in the thigh muscle. C1 (control group 1) was injected with normal saline at 0.9% intramuscularly for 14 days. C2 (control group 2) was injected with normal saline at 0.9% intramuscularly for 21 days [27]. After 24 hours of last injections, the

animals were prepared for abdominal dissection. The animals were anesthetized with chloroform, then abdominal dissection was done and pancreas obtained; after that, the gland was fixed with 10% buffered formalin for 24 hours, dehydration was done, clearance was done, embedding in paraffin was obtained, blocks were obtained, and 4 µm sections were prepared for immunohistochemical staining for each marker.

Immunohistochemistry

A 4 µm thickness of two serial sections was taken from each block. for immunohistochemical staining, these sections were positioned on positively charged slides with anti-BAX and anti-eNOS primary antibodies (Santa Cruz®). The secondary antibodies were mouse-specific HRP/DAB (Abcam), code ab64261. The sections were dewaxed with xylene and then hydrated. Antigen retrieval was done for 20 minutes using pressure cooking with citrate buffer. The delusion for primary antibodies was 1:200 by diluent buffer from Abcam® (Ab64211) and maintained for 30 minutes at room temperature. After that, by using labeled streptavidin biotin from the secondary detection kit, the detection was done. Finally, DAB and chromogen staining were performed. Then slides were counterstained with hematoxylin and fixed with DPX [28]. The assessments were done blindly, with the detection of anti-BAX and anti-eNOS when the nucleus and cytoplasm were stained with brown staining. The slides were examined at 10_X (low power) to determine the areas of high staining; if they did not show any stain, they would be examined at 40X (high power) to find the areas of weak staining [29]. Five fields were taken for each section, and scoring was done semi-quantitatively by finding the ratio of cells stained positively (nuclei or cytoplasm) over the total number of the assessed cells. The intensity of positive cells was assessed at four levels, from 0 to 3+. The total score was obtained by multiplying the percentage of positive cells by the intensity of staining [30]. The staining intensity score was 0 for negative staining, 1+ for cells with weak staining, 2+ for cells with moderate staining, and 3+ for cells with strong staining. The staining percentage was 0 for negative staining cells, 1 when <10% of total cells stained positively, 2 when 10–50% of total cells stained positively, and 3 when >50% of cells stained positively. The examination was done using a double-blind method and was confirmed using a computerized method (Immunohistochemical Profiler Plugin and Macro in image J).

Data analysis

Data were analyzed using the available statistical packages of IBM SPSS-26 (2019) and Microsoft Excel 2023 (Microsoft Family 360). Data are presented as mean ± SD and tested by a student t-test and an ANOVA test. The statistical significance was tested at a *p-value of* <0.05 with a 95% confidence interval.

RESULTS

As shown in Table 1, there was no statistical difference between the control groups for long and short durations when we compared the weight of the rabbit in weeks 1 through 3, where the *p*-value was 0.47 and 0.35, respectively.

Table 1: The mean changes in weight of the rabbits during different time intervals

Groups	Weight (g)
GS1WK1	1000±136.9a
GS1WK2	1135±131.81c
GS2WK1	960±96.177a
GS2WK2	1132±128.33c
GL1WK1	1060±129.42a
GL1WK3	1360±80.312b
GL2WK1	990±174.64a
GL2WK3	1404±127.78b
C1WK1	1014±112.8a
C1WK2	1040±114a
C2WK1	1030±120.4a
C2WK3	1061±129.8a

Values were expressed as mean±SD. Values with different superscripts (a,b,c) among groups are significantly different ($p<0.05$).

The female rabbit that was treated with 5 mg/kg of hydrocortisone for 21 days (GL1) and the female rabbit that was injected with 20 mg/kg of hydrocortisone for 21 days (GL2) showed an increase in weight by the end of the 3rd week. There was a high statistical difference when comparing GL1 in the first and third weeks, where the *p*-value was 0.001, and also for GL2, where the *p*-value was 0.002 (Figure 1).

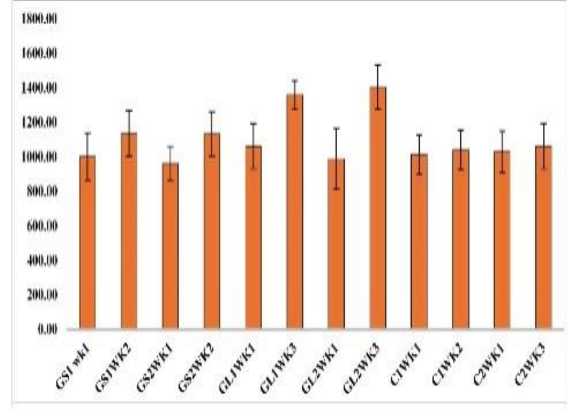

Figure 1: The weight (g) of female rabbits throughout weeks 1, 2 and 3 in GS and GL duration of hydrocortisone injection.

There was no statistical difference in both short-duration groups that were injected with 5 mg/kg and 20 mg/kg of hydrocortisone throughout weeks 1 and 2 (*p*-value= 0.84 and 0.83, respectively). Also, there was no statistical difference when we compared the GS1 and GL1 in week 1, but when we compared them in the third week, there was a highly statistical difference where the *p*-value was 0.002. There was a significant increase in the expression of BAX in GS1 and GS2 when compared to the control group (*p*-value= 0.001) (Table 2).

Table 2: expression of BAX after short duration of injection groups

Groups	Bax level
GS1	1.66±0.33
GS2	2.18±0.46
C1	0.20±0.07
C2	0.164±0.06
p-value	0.001

Values were expressed as mean±SD.

BAX expression was higher in GS2 than in GS1, but it was not significant (*p*-value= 0.06). GL1 and GL2 groups expressed BAX significantly higher than that of the control (*p*-value= 0.001). The GL1 group expressed BAX significantly more than that of GL2 (*p*-value= 0.03) (Table 3).

Table 3: Expression of BAX in GL1 and GL2 compared to control group

Groups	Bax level
GL1	2.58±0.29
GL2	1.54±0.41
C1	0.20±0.07
C2	0.164±0.06
p-value	0.001

Values were expressed as mean±SD.

GS1 and GS2 expressed eNOS significantly higher than the control group (*p*-value= 0.001), and it was significantly higher in GS2 (*p*-value= 0.001) (Table 4).

Table 4: Expression of eNOS in GS1 and GS2 compared to control group

Groups	eNOS level
GS1	2.5±0.4
GS2	2.9±0.1
C1	0.41±0.16
C2	0.24±0.11
p-value	0.001

Values were expressed as mean±SD.

Both the GL1 and GL2 groups expressed eNOS significantly higher when compared to the control group of the same duration (*p*-value= 0.001). There was no statistical difference between GL1 and GL2 (*p*-value= 0.07) (Table 5).

Table 5: Expression of eNOS in GL1 and GL2 compared to control group

Groups	eNOS level
GL1	2.4±0.2
GL2	2.2±0.2
C1	0.41±0.16
C2	0.24±0.11
p-value	0.001

Values were expressed as mean±SD.

Also, there was no statistical difference when we compared the different groups with the same duration as GS1 and GL1, where the P value was 0.6. Nevertheless, eNOS was expressed in GS2 more than GL2 did (*p*-value= 0. 001). The changes in BAX and eNOS expression in all groups were demonstrated in Figure 2.

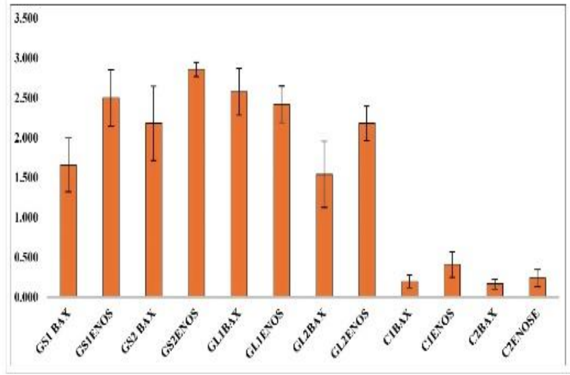

Figure 2: Expression of BAX and eNOS among groups of female rabbits injected with multiple doses of hydrocortisone in different durations.

Furthermore, Figure 3 displays microphotographs depicting the expression of BAX and eNOS in the pancreas of female rabbits that were injected with hydrocortisone at doses of 5 mg and 20 mg for varying durations.

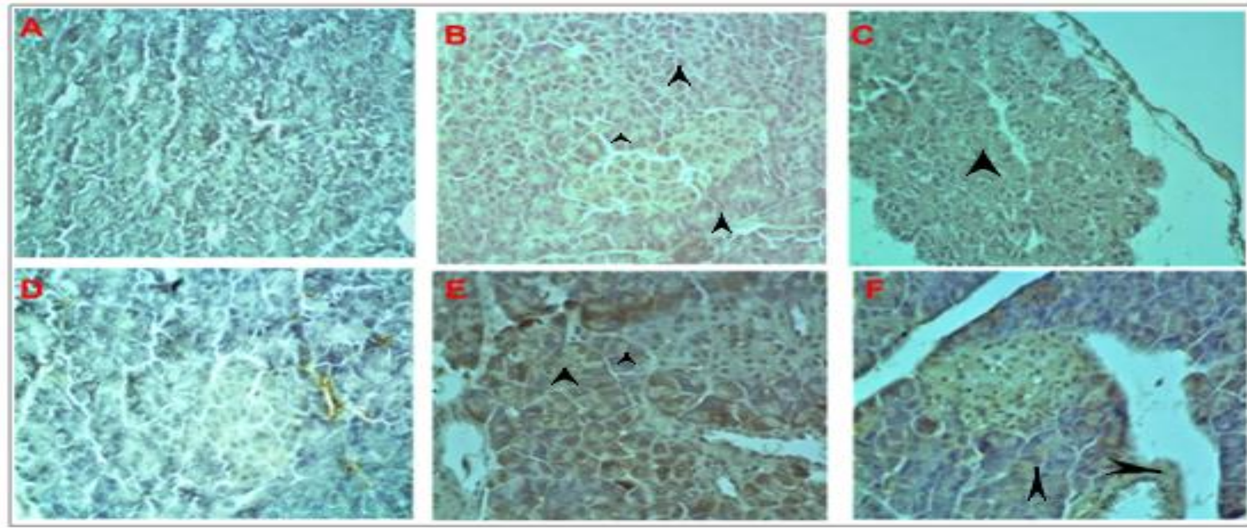

Figure 3: Microphotographs of BAX and eNOS expression in pancreas in female rabbits injected with hydrocortisone (5 mg and 20 mg) for two- and three-weeks (A). BAX expression in control group (negative expression) (B). BAX expression in female rabbit pancreas after 2 weeks (C). BAX expression after 3 weeks (D). eNOS expression in control group (E). eNOS expression after 2 weeks (F). eNOS expression after 3 weeks (black arrows in B, C, E, and F).

DISCUSSION

Glucocorticoids are considered crucial for body metabolism, and since hydrocortisone has glucocorticoid and mineralocorticoid actions, changes in water balance are obtained when hydrocortisone is used. In addition, an increase in energy intake occurs because of hydrocortisone's stimulatory effect on the central appetite centers. All these effects explain the weight gain that we obtained in this study, and it is in accordance with the results obtained by other researchers, who also claimed that this weight gain is dose- and duration-dependent [31]. On the other hand, the pancreas needs glucocorticoids to safeguard its structural integrity [32]. Though the dosage and duration of these glucocorticoids' effects on the pancreas anatomy and function must be considered [33], it is crucial to note that an appropriate glucocorticoid dose stimulates acinar cell activity, whereas an excessive dose reduces exocrine tissue secretory activity and impairs its ability to synthesize protein [34]. The activation of acinar cell functions therefore requires physiological amounts of glucocorticoids, whereas steroid excesses have an inhibitory impact on acinar cells and cause degeneration to develop [35]. Pancreas-specific proteins are stimulated to be produced by glucocorticoids, most likely through a cytoplasmic-nuclear receptor pathway [36]. These corticosteroids appear to either encourage the accumulation of certain RNAs or increase the transcription of RNAs from gene loci closely linked to proteins particular to the pancreas [37]. Therefore, it appears that glucocorticoids are crucial for maintaining a healthy level of exocrine enzymes in the developing embryonic pancreas [38], in addition to the mature gland [39]. However, it should be underlined that the regulating impact of corticosteroids in intact animals can occur at extremely low concentrations (10–8 to 10–6 M) [40]. In contrast, greater amounts of these hormones appear to have a negative impact on the ability of the pancreatic acinar cells to synthesize new cells, resulting in an injured pancreas. The GL2 group in this study showed a decrease in BAX expression. So, the BAX expression decreased with an increase in the duration of the high dose of injection. So, the expression of BAX depended on the dose of hydrocortisone. But the eNOS expression didn't depend on the dose of hydrocortisone injected. BAX is one of the crucial proteins that control mitochondrial permeability and apoptotic signaling [41]. As a result, the amount of mitochondrial cytochrome c that is released into the cytosol to interact with the apoptotic peptidase activating factor and create the apoptosome, which then activates caspase 9 [42], ultimately causing cell death, is increased [43]. It has been shown that hydrocortisone increased the level of the protein BAX and decreased the level of the anti-apoptotic protein Bcl2, demonstrating that hydrocortisone triggered mitochondrial-mediated apoptosis, which is considered one of many other pathways of apoptosis that is triggered by glucocorticoids, and that is what we obtained in our study after treatment with hydrocortisone for 3 weeks and by using a high dose of the drug exclusively [44]. The hydrocortisone effect on the pancreas is stimulatory first, and therefore, the production of proteins from the exocrine pancreas is increased, but this is dose- and duration-related, and therefore, as the dose is increased to 20 mg/kg and the duration lengthens to 3 weeks, the protein synthesis is depleted and the cells become exhausted, and the features will be associated with the appearance of the degenerated cells. This can be observed by the statistically significant results of BAX protein expression, which is a marker for cell apoptosis. This will give us the idea that the cells will shift from actively protein-synthesizing cells to degenerated, exhausted cells [45]. Numerous alterations in eNOS mRNA and protein levels, eNOS intracellular location, eNOS interaction with regulatory proteins in the signaling complex, and phosphorylation of serine, threonine, and tyrosine residues are all factors that affect how eNOS is regulated. Even though many of these stages have been thoroughly explained in in vitro models and in cell lines that overexpress one or more members of the signaling complex, much more research is necessary to ascertain which alteration controls eNOS activity in native tissues [46]. The endothelial NO synthase (eNOS) is activated in response to fluid shear stress and various agonists through cellular processes such as increased intracellular Ca^{2+}, interaction with substrates and co-factors, as well as adaptor and regulatory proteins, protein phosphorylation, and shuttling between different sub-cellular domains [47]. Numerous pathophysiological conditions, including diabetes and atherosclerosis, have lowered NO output and attenuated eNOS activity because of dysregulation of these processes [48]. Glucocorticoids also have a vasoconstriction effect on the endothelial cells of the body and tissues, and this also contributes to the injury of the pancreatic tissue using hydrocortisone, which is also dose- and duration-related. This finding is supported by the work of other researchers in this field [49]. All these injuries that the pancreas was subjected to when a high and long steroid duration injection occurred led to the elaboration of NO and a rise in the pancreatic tissue's eNOS expression in the treated animals, which indicates a pancreatic injury.

Study limitations

One potential drawback of the current study is the exclusion of male rabbits and female rabbits who are younger than seven months old and pregnant females.

Conclusions

Extended use of hydrocortisone beyond two weeks can cause damage to pancreatic tissues, regardless of the dosage administered.

Conflict of interests

No conflict of interests was declared by the authors.

Funding source

The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Alam MM, Okazaki K, Nguyen LTT, Ota N, Kitamura H, Murakami S, et al. Glucocorticoid receptor signaling represses the antioxidant response by inhibiting histone acetylation mediated by the transcriptional activator NRF2. *J Biol Chem*. 2017;292(18):7519–7530. doi: 10.1074/jbc.M116.773960.
2. Wang M, Jiang Z, Liang H. Glucocorticoids in acute pancreatitis: a propensity scores matching analysis. *BMC Gastroenterol*. 2021;21:331. doi: 10.1186/s12876-021-01907-1.
3. Chan ED, Chan MM, Chan MM, Marik PE. Use of glucocorticoids in the critical care setting: Science and clinical evidence. *Pharmacol Ther*. 2020;206:107428. doi: 10.1016/j.pharmthera.2019.107428.
4. Meduri GU, Bridges L, Shih MC, Marik PE, Siemieniuk RAC, Kocak M. Prolonged glucocorticoid treatment is associated with improved ARDS outcomes: analysis of individual patients' data from four randomized trials and trial-level meta-analysis of the updated literature. *Intensive Care Med*. 2016;42(5):829–840. doi: 10.1007/s00134-015-4095-4.
5. Ledford H. Coronavirus breakthrough: dexamethasone is first drug shown to save lives. *Nature*. 2020;582(7813):469. doi: 10.1038/d41586-020-01824-5.
6. Wang K, Tan F, Zhou R, Liu D, Ni Z, Liu J, et al. Therapeutic response to corticosteroids in a critically ill patient with COVID-19: A case report. *Medicine*. 2020;99(31):e21597. doi: 10.1097/MD.0000000000021597.
7. Ataallah B, Abdulrahman M, Al-Zakhari R, Buttar BS, Nabeel S. Steroid-induced pancreatitis: A challenging diagnosis. *Cureus*. 2020;12(7):e8939. doi: 10.7759/cureus.8939.
8. Sadr-Azodi O, Mattsson F, Bexlius TS, Lindblad M, Lagergren J, Ljung R. Association of oral glucocorticoid use with an increased risk of acute pancreatitis: a population-based nested case-control study. *JAMA Intern Med*. 2013;173:444–449. doi: 10.1001/jamainternmed.2013.2737.
9. Overman RA, Yeh JY, Deal CL. Prevalence of oral glucocorticoid usage in the United States: a general population perspective. *Arthritis Care Res (Hoboken)*. 2013;65(2):294-298. doi: 10.1002/acr.21796.
10. Makol A, Petri M. Pancreatitis in systemic lupus erythematosus: frequency and associated factors—a review of the Hopkins Lupus Cohort. *J Rheumatol*. 2010;37(2):341-345. doi: 10.3899/jrheum.090829.
11. Chawla S, Atten MJ, Attar BM. Acute pancreatitis as a rare initial manifestation of Wegener's granulomatosis: a case based review of literature. *JOP*. 2011;12(2):167-169. PMID: 21386646.
12. Kandil E, Lin YY, Bluth MH, Zhang H, Levi G, Zenilman ME. Dexamethasone mediates protection against acute pancreatitis via upregulation of pancreatitis-associated proteins. *World J Gastroenterol*. 2006;12(42):6806-6811. doi: 10.3748/wjg.v12.i42.6806.
13. Zhang XP, Chen L, Hu QF, Tian H, Xu RJ, Wang ZW, et al. Effects of large dose of dexamethasone on inflammatory mediators and pancreatic cell apoptosis of rats with severe acute pancreatitis. *World J Gastroenterol*. 2007;13(41):5506-5511. doi: 10.3748/wjg.v13.i41.5506.
14. Sugiyama Y, Kato S, Abe M, Mitsufuji S, Takeuchi K. Different effects of dexamethasone and the nitric oxide synthase inhibitor L-NAME on caerulein-induced rat acute pancreatitis, depending on the severity. *Inflammopharmacology*. 2005;13:291–301. doi: 10.1163/156856005774423728.
15. Paszt A, Takács T, Rakonczay Z, Kaszaki J, Wolfard A, Tiszlavicz L, et al. The role of the glucocorticoid-dependent mechanism in the progression of sodium taurocholate-induced acute pancreatitis in the rat. *Pancreas*. 2004;29:75-82. doi: 10.1097/00006676-200407000-00059.
16. Noel KI. Hepatic tissues under the effect of dexamethasone: Histological study, dose and duration related changes. *Iraqi J Med Sci*. 2013;11(2):113-118.
17. Wang ZF, Pan CE, Lu Y, Liu SG, Zhang GJ, Zhang XB. The role of inflammatory mediators in severe acute pancreatitis and regulation of glucocorticoids. *Hepatobiliary Pancreat Dis Int*. 2003;2:458-462. PMID: 14599960.
18. Liu JS, Wei XG, Fu J, Liu J, Yuan YZ, et al. Stady of the relationship among endothelin, nitric oxide, oxgen free radical and acute pancreatitis. *Zhongguo Yishi Zazhi*. 2003;5:28–29.
19. Dong R, Wang ZF, LV Y, Ma QJ. Treatment of severe acute pancreatitis with large dosage of dexamethasone in the earlier time. *Gandan Waike Zazhi*. 2005;13:58–60.
20. Wang ZF, Liu C, Lu Y, Dong R, Xu J, Yu L, et al. Dexamethasone and dextran 40 treatment of 32 patients with severe acute pancreatitis. *World J Gastroenterol*. 2004;10:1333-1336. doi: 10.3748/wjg.v10.i9.1333.
21. Al-Kaabi M, Noel K, Al-Rubai A. Evaluation of immunohistochemical expression of stem cell markers (NANOG and CD133) in normal, hyperplastic, and malignant endometrium. *J Med Life*. 2022;15(1):117-123. doi: 10.25122/jml-2021-0206.
22. Kandil E, Lin Y, Levy G, Bluth M, Zenilman M. Dexamethasone mediates protection against acute pancreatitis in rats via upregulation of pancreatitis of pancreatitis-associated protein (PAP). *Gastroenterology*. 2004;126:A781. doi: 10.3748/wjg.v12.i42.6806.
23. Campbell PD, Weinberg A, Chee J, Mariana L, Ayala R, Hawthorne WJ, et al. Expression of pro- and antiapoptotic molecules of the Bcl-2 family in human islets post isolation. *Cell Transplant*. 2012;21(1):49-60. doi: 10.3727/096368911X566262.
24. Frossard JL, Quadri R, Hadengue A, Morel P, Pastor CM. Endothelial nitric oxide synthase regulation is altered in pancreas from cirrhotic rats. *World J Gastroenterol*. 2006;12(2):228-233. doi: 10.3748/wjg.v12.i2.228.
25. Nikolaou N, Hodson L, Tomlinson JW. The role of 5-reduction in physiology and metabolic disease: evidence from cellular, pre-clinical and human studies. *J Steroid Biochem Mol Biol*. 2021;207:105808. doi: 10.1016/j.jsbmb.2021.105808.
26. Al-Mosawi RH, Alebadi NAM. Usage of corticosteroids as therapeutic agents in diseases. *Med J Babylon*. 2023;20:447-450. doi: 10.4103/MJBL.MJBL_391_23.
27. Matthes H, Kaiser A, Stier U, Riecken EO, Rosewicz S. Glucocorticoid receptor gene expression in the exocrine and endocrine rat pancreas. *Endocrinology*. 1994;135(1):476-479. doi: 10.1210/endo.135.1.8013388.
28. Hameed AF, Noel KI, Akkila SS. Placental angiogenesis, IUGR & CMV awareness in Iraqi women. *Curr Issues Pharm Med Sci*. 2022;35(3):147-151. doi: 10.2478/cipms-2022-0027.
29. Noel KI, Ibraheem MM, Ahmed BS, Hameed AF, Khamees NH, Akkila SS. Expression of OCT4 stem cell marker in benign prostatic hyperplasia and normal tissue around the prostatic carcinoma in a sample of Iraqi patients. *Egypt J Histol*. 2020;43(1):245-254. doi: 10.21608/EJH.2019.13791.1130.
30. Noel KI, Ibraheem MM, Ahmed BS, Hameed AF, Khamees NH, Akkila SS. CD133 and CD166 expression predicting the possibility of prostatic cancer development in cases of BPH. *Biomed Pharmacol J*. 2019;12(3):1403-1416. doi: 10.13005/bpj/1769.
31. Bannon CAM, Border D, Hanson P, Hattersley J, Weickert MO, Grossman A, et al. Early metabolic benefits of switching hydrocortisone to modified release hydrocortisone in adult adrenal insufficiency. *Front Endocrinol (Lausanne)*. 2021;12:641247. doi: 10.3389/fendo.2021.641247.
32. Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med*. 2016;48(3): e219. doi: 10.1038/emm.2016.6.
33. Nomoto H, Gurlo T, Rosenberger M, Girgis MD, Dry S, Butler PC. Low grade islet but marked exocrine pancreas inflammation in an adult with autoimmune pre-diabetes. *Case Rep Endocrinol*. 2019;2019:5863569. doi: 10.1155/2019/5863569.
34. Guo L, Lichten LA, Ryu MS, Liuzzi JP, Wang F, Cousins RJ. STAT5-glucocorticoid receptor interaction and MTF-1 regulate the expression of ZnT2 (Slc30a2) in pancreatic acinar cells. *Proc Natl Acad Sci U S A*. 2010;107(7):2818-2823. doi: 10.1073/pnas.0914941107.
35. Zhang J, Ge P, Liu J, Luo Y, Guo H, Zhang G, et al. Glucocorticoid treatment in acute respiratory distress syndrome: An overview on mechanistic insights and clinical

benefit. *Int J Mol Sci*. 2023;24(15):12138. doi: 10.3390/ijms241512138.
36. Vegiopoulos A, Herzig S, Glucocorticoids, metabolism and metabolic diseases. *Mol Cell Endocrinol*. 2007;275(1-2):43-61. doi: 10.1016/j.mce.2007.05.015.
37. Cutroneo KR, Sterling KM. How do glucocorticoids compare to oligo decoys as inhibitors of collagen synthesis and potential toxicity of these therapeutics? *J Cell Biochem*. 2004;92:6-15. doi: 10.1002/jcb.20030.
38. Esguerra JLS, Ofori JK, Nagao M, Shuto Y, Karagiannopoulos A, Fadista J, et al. Glucocorticoid induces human beta cell dysfunction by involving riborepressor GAS5 LincRNA. *Mol Metab*. 2020;32:160-167. doi: 10.1016/j.molmet.2019.12.012.
39. Bockman DE, Guo J, Buchler P, Muller MW, Bergmann F, Friess H. Origin and development of the precursor lesions in experimental pancreatic cancer in rats. *Lab Invest*. 2003;83:853-859. doi: 10.1097/01.lab.0000074918.31303.5a.
40. Saleh A, Srinivasula SM, Acharya S, Fishel R, Alnemri ES. Cytochrome c and dATP-mediated oligomerization of Apaf-1 is a prerequisite for procaspase-9 activation. *J Biol Chem*. 1999;274:17941–17945. doi: 10.1074/jbc.274.25.17941.
41. Smith LK, Cidlowski JA. Glucocorticoid-induced apoptosis of healthy and malignant lymphocytes. *Prog Brain Res*. 2010;182:1-30. doi: 10.1016/S0079-6123(10)82001-1.
42. Herr I, Gassler N, Friess H, Buchler MW. Regulation of differential pro- and anti-apoptotic signaling by glucocorticoids. *Apoptosis*. 2007;12:271-291. doi: 10.1007/s10495-006-0624-5.
43. Gruver-Yates AL, Cidlowski JA. Tissue-specific actions of glucocorticoids on apoptosis: a double-edged sword. *Cells*. 2013;2(2):202-223. doi: 10.3390/cells2020202.
44. Hashish HA. Effect of metformin on Bax expression in pancreas and testis of normoglycemic albino rat: An immunohistochemical study. *Indian J Clin Anat Physiol*. 2020;7(2):134-141. doi: 10.18231/j.ijcap.2020.028.
45. Shah V, Cao S, Hendrickson H, Yao J, Katusic ZS. Regulation of hepatic eNOS by caveolin and calmodulin after bile duct ligation in rats. *Am J Physiol Gastrointest Liver Physiol*. 2001;280:G1209–G1216. doi: 10.1152/ajpgi.2001.280.6.G1209.
46. Fleming I. Molecular mechanisms underlying the activation of eNOS. *Pflugers Arch*. 2010;459(6):793-806. doi: 10.1007/s00424-009-0767-7.
47. Ahmed TH, Mshimesh BAR, Raoof IB. Effect of nicorandil on endothelial markers and tissue remodeling in pulmonary arterial hypertension model of male rats. *Al-Rafidain J Med Sci*. 2023;5(1S):S87-93. doi: 10.54133/ajms.v5i1S.334.
48. Lei Ying, Hofseth LJ. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. *Cancer Res*. 2007;67(4):1407-1410. doi: 10.1158/0008-5472.CAN-06-2149.
49. Araujo JEDS, Miguel-Dos-Santos R, Macedo FN, Cunha PS, Fontes MT, Murata GM, et al. Effects of high doses of glucocorticoids on insulin-mediated vasodilation in the mesenteric artery of rats. *PLoS One*. 2020;15(3):e0230514. doi: 10.1371/journal.pone.0230514.