Metformin inhibits polyphosphate-induced hyper-permeability and inflammation

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2021-10

International Immunopharmacology

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--Manuscript Draft--

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Running title: Metformin inhibits polyP-induced inflammation

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Abstract

Circulating inflammatory factor inorganic polyphosphate (polyP) released from activated platelets could enhance factor XII and bradykinin resulted in increased capillary leakage and vascular permeability. PolyP induce inflammatory responses through mTOR pathway in endothelial cells, which is being reported in several diseases including atherosclerosis, thrombosis, sepsis, and cancer. Systems and molecular biology approaches were used to explore the regulatory role of the AMPK activator, metformin, on polyP-induced hyper-permeability in different organs in three different models of polyP-induced hyper-permeability including local, systemic short- and systemic long-term approaches in murine models. Our results showed that polyP disrupts endothelial barrier integrity in skin, liver, kidney, brain, heart, and lung in all three study models and metformin abrogates the disruptive effect of polyP. We also showed that activation of AMPK signaling pathway, regulation of oxidant/anti-oxidant balance, as well as decrease in inflammatory cell infiltration constitute a set of molecular mechanisms through which metformin elicits it's protective responses against polyP-induced hyper-permeability. These results support the clinical values of AMPK activators including the FDA-approved metformin in attenuating vascular damage in polyP-associated inflammatory diseases.

Keywords: AMPK signaling, Inorganic polyphosphate, Metformin, Vascular permeability

Introduction

Inflammation relies on the immune and tissue responses by recruiting immune cells and related mediators [1]. The circulating inflammatory factor inorganic polyphosphate (polyP) enhances the recruitment of chemotactic agents, trigger the leukocytes motility, over-express cell surface adhesion molecules, and disturb vascular permeability [2]. PolyP is a linear anionic polymer with a simple structure linked by phosphoanhydride bonds [3]. PolyP is ubiquitously synthesized in various organisms and tissues with different length from 3 to over 1,000 phosphate residues [4, 5]. Extensive studies on polyP biosynthesis have indicated that polyP is enzymatically synthesized from adenosine triphosphate (ATP) in microorganisms [6]. In this process the γ-phosphate is released from ATP and delivered to terminal site of polyP polymer which is catalyzed by polyphosphate kinase (PPK) [6]. In mammalian cells, polyP is found in mitochondria, lysosomes, nuclei, and also in granules of platelets, mast cells and basophils [7]. In human serum or plasma, polyP has a half-life of approximately 1.5 to 2 hours and degraded by exopolyphosphatase and/or endopolyphosphatase [8, 9].

Polyphosphate regulates energy metabolism [10], bone mineralization [11], cell growth [12, 13], tumor invasiveness [14, 15], coagulation [16-22] and inflammation [2]. It is known that polyP stimulates inflammatory responses by activating the contact pathway [23] regulating complement system [24], activating nuclear factor-kappa B (NF-κB) [25].

Furthermore, it has been shown that polyP released from activated platelets can induce FXII and bradykinin resulted in increased capillary leakage [26]. Bradykinin as one of the main downstream effectors of polyP, induces various cellular signaling cascades that dilate vessels, and increases vascular permeability [27]. Additionally, FXIIa can activate the complement system leading to accumulation of C3a and C5a, that promote permeability in host-defense reactions [28, 29].

To explore the function of polyP in pathogenesis of human diseases it has also been reported that polyP strongly promoted the amyloid formation of several amyloidogenic proteins

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like β2-Microglobulin which is associated with multiple human disorders like Alzheimer, Parkinson, and dialysis-related amyloidosis [30, 31]. Moreover, previous studies have established an important role for polyP in promoting inflammatory responses in endothelial cells resulted in multiple human diseases including sepsis, septic shock, atherosclerosis, thrombosis, and cancer [32-35].

polyP induces inflammatory responses in endothelial cells trough binding to the RAGE and P2Y1 receptors resulted in phosphorylation and inactivation of TSC (tuberous sclerosis complex) proteins as master regulator of the mTOR (mammalian target of rapamycin) signaling pathway. Consistent with these findings, we previously showed that polyP regulates inflammation through up-regulation of the mammalian target of rapamycin (mTOR) signaling pathways in vascular system [36]. Furthermore, it has been shown that mTOR signaling is repressed via the activation of AMP-activated protein kinase (AMPK), a major modulator of cellular energy homeostasis [37-39].

Metformin, initially identified as a biguanide antibiotic drug, has been listed as a potent AMPK activator [40], down-regulating anti-inflammatory responses through activating AMPK [41, 42]. It has been shown that metformin down-regulates expression of pro-inflammatory cytokines and cell adhesion molecules by activating AMPK in vascular endothelial cells [43]. Metformin also inhibits eosinophilic inflammation, and reduces oxidative stress [44] by up-regulating expression of anti-oxidant molecules and decreasing intestinal reactive oxygen species (ROS) [45].

In this study we investigated protective effects of metformin on polyP-induced hyper-permeability in three different models which probe the local, systemic short- and long-term inflammatory effects of polyP in different organs of mice. Our results showed that metformin significantly abrogates vascular leakage and inflammation in polyP-stimulated [40] mice at least partially via activation of the AMPK pathway, inhibition of oxidative stress, and inhibition of the

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pro-inflammatory cell infiltration to the damaged vasculature, suggesting the therapeutic potential of this drug in preventing vascular leakage in polyP-mediated inflammatory diseases.

Material and Methods

Materials

PolyP-700 was purchased from Kerafast Inc. (Boston, MA). Evans Blue (EB) and formamide were obtained from Merck (Darmstadt, Germany). Metformin, dorsomorphin, Haematoxylin and Eosin (H&E), Penicillin, streptomycin, as well as all reagents needed for measuring malonyl dialdehyde (MDA), superoxide dismutase (SOD), catalase, and total thiol were all purchased from Sigma Co (Saint Louis, MO). F12/Dulbecco's Modified Eagle Medium (DMEM/F12) and fetal bovine serum (FBS) were obtained from Gibco Laboratories (Grand Island, NY). RIPA lysis buffer and BCA protein assay kit were from Thermo Scientific (Rockford, IL).

Bioinformatic analysis of the polyP-interacting proteome affected by metformin

The list of proteins in the human proteome that interact with polyP were extracted from data provided by Azevedo et al. [46]. Briefly, in this study, authors have generated polyP species labeled with biotin and used them to screen 15000 proteins in a Human Proteome Array slide interacting with it. With application of two screening procedures by modifying the concentration of polyP and the buffer pH, they identified 74 overlapping proteins in the two procedures. These authors then performed Gene Ontology (GO)-based enrichment analysis using Panther database using the array proteins as the reference set [47]. We thus used the 74 protein list and performed pathway enrichment analysis using the whole proteome as the reference in the EnrichR database [48]. Adjusted p-value of <0.05 was considered significant.

In order to decipher the underlying mechanism of neutralizing polyP effects on cellular processes by metformin, exemplar gene expression changes induced by this drug in various cell types were extracted from datasets (LINCSCP_20754, LINCSCP_21432, LINCSCP_272348,

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LINCSCP_27336, LINCSCP_43496 and LINCSCP_4) deposited in iLINCS database [49, 50]. To extract the most representative metformin induced gene signature from different cell lines, Principal Component Analysis (PCA) was performed on the signatures. Cell lines with the least deviations were HT29, HCC and PC3. From these cellular signatures, overlapping genes with significant alterations in at least 2 cell lines (adjusted p-value < 0.05 and -0.5 < log(FC)<0.5 were selected for pathway enrichment. An additional dataset from a study by Bentley-DeSousa et al. was also assessed to identify potential targets of polyP that contain PASK motif (Poly acidic, Serine (S) and Lysine (K) residue) that overlap with differentially expressed genes by metformin [51]. Pheatmap package in R was used to generate heatmap of expression data [52].

Cell culture

Immortalized HUVECs (EA.hy926) cell line was obtained from Pasteur Institute (Tehran, Iran). Cells were cultured in DMEM/F12 medium supplemented with 10% heat-inactivated FBS and 1% streptomycin/penicillin at 37°C under a humidified atmosphere of 5% CO_2.

Animal and Compliance with ethics guidelines

The protocols for the animal studies were approved by the ethical committee and conducted based on the guideline for Care and Use of Laboratory Animals from Mashhad University of Medical Sciences (MUMS). Male BALB/c mice were obtained from Pasteur Institute (Tehran, Iran) and maintained under standard environmental conditions. Mice were housed at room temperature (20-25 ºC) and 12 h light/dark cycle with free access to food and water ad libitum.

Permeability assays

The polyP-induced hyper-permeability was performed according to three different models including local (50-200 μg polyP/mouse) administered intra-dermally (id), systemic short-term (4-400 μg polyP/mouse injected in the intraperitoneal cavity (ip), and systemic long-term (4 and 40 μg polyP/mouse/day ip) [15, 36]. In each model, mice were divided into four groups: 1) control group, mice received normal saline, 2) polyP group, 3) metformin/polyP

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group, and 4) Dorsomorphin/Metformin/polyP group. To visualize vascular permeability in different groups, the Evans Blue (EB) dye (20mg/ml) was directly injected into the tail vein of anesthetized mice intravenously (iv) and tissues were isolated and maintained in formamide solution. The polyP-triggered leakage of of the albumin bound EB dye to different organs was monitored by a spectrophotometer at 620 nm as described previously [53].

Histological staining

The harvested tissues were fixed in formalin, embedded in paraffin, and sectioned using a microtome. The de-paraffinzed tissue sections were mounted and stained with haematoxylin and eosin (H&E) or Trichrome for the histologic assessment.

Evaluation of oxidative stress markers

The extent of the oxidative stress was evaluated via measuring the level of MDA, total thiol, catalase and superoxide dismutase (SOD) activities in the tissue homogenates as described [54]. Intracellular reactive oxygen species (ROS) generation was analyzed using 2', 7'-dichlorodihydrofluorescein diacetate (DCFDA) cellular reactive oxygen species detection assay kit according to the manufacturer's instructions (Abcam, Cambridge, MA) [55].

Statistical analysis

All data were expressed as mean values ± Standard Error (SE). P value of less than 0.05 was considered as statistically significant. Statistical comparisons were determined using Student's t-test or One-way ANOVA for parametric data followed by Tukey's multiple comparisons test.

Results

In-silico analyses show that polyP affects inflammation through modulation of the proteasome pathway

PolyP has been shown to affect various pathways in eukaryotes including hemostasis and inflammation; however the exact underlying mechanism is not yet fully understood.

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Recently, an interesting high-throughput study was performed to identify the subset of proteome that interacts with polyP [46]. We thus used the list of 74 proteins publically provided by these authors and performed pathway enrichment analyses on the gene set using EnrichR web tool. We observed that the most significantly enriched pathway was related to the proteasome and ubiquitin pathways (adjusted p-value < 0.05). The genes leading to enrichment of these pathways were various components of proteasome subunits and heat shock proteins (Fig. 1A). Consistently, using the EnrichR web tool, the enriched GO biological processes were activation of innate immune response, activation of NF-kB pathway as well as negative regulation of cell cycle, which are known to be associated with proteasome pathway (Fig. 1B).

In silico data suggest that metformin abrogates the cellular effects of polyp

The enriched pathways and biological processes suggested that polyP elicits inflammation at least partially by NF-κB activation which is consistent with the literature [25]. Moreover, we have shown that NF-κB and mTORC1 pathways are both regulated during the polyP-induced inflammatory processes [36]. Thus, we hypothesized that metformin, as an AMPK activator, may exert protective effects against polyP-mediated NF-κB activation and inflammation. We initially analyzed the metformin-induced changes in gene expression using the iLINCS database. Among datasets, we found 6 exemplar signatures in various cell lines. To exclude the cell context effect on analyzing the effect of metformin, we performed a PCA test on gene expression changes in the 6 cell lines treated with metformin. We observed that alterations in HCC, PC3 and HT29 were the most similar and thus chose gene expression changes in these 3 cell lines for further analyses (Figure 1C). We observed that from the polyp interacting protein list obtained from Azevedo et al study, the expression of Abhydrolase Domain Containing 4 (ABHD4), RuvB-like protein (RUVBL1), and polynucleotide phosphorylase (PNP) were significantly decreased by metformin in at least 2 of the 3 assessed cell lines (Figure 1D). Moreover, polyP is known to interact with poly acidic proteins containing PASK domain ([Poly-Acidic serine (S) and lysine (K) rich) and the list of candidate proteins containing such PASK-

like motifs has been recently published [51]. We found that 9 proteins with PASK motif are differentially expressed following metformin treatment (Figure 1E).On the other hand, pathway enrichment analysis using KEGG and WikiPathways on metformin-induced alterations in the gene expression profiles showed that metformin significantly regulates apoptosis, oxidative responses, PI3K/Akt and AMPK signaling pathways which are also targets for polyP. These results suggest that metformin exerts its protective effect through modulation of the expression of the same proteins that are targets for the pro-inflammatory function of polyP.

polyP induces hyper-permeability in local and systemic short-and long-term models

Inflammation is an important modulator of the vascular leakage. To determine the dose-dependent effects of polyP treatment on permeability, we assessed whether increased concentration of polyP (50-200 µg/mouse id) could change the permeability in a local administration method (Fig 2A). Our results showed that polyP increased vascular leakage in local group (Fig. 3A). PolyP at a concentration of 50 µg/mouse significantly elevated permeability in skin (P<0.05). Interestingly, lower permeability was detected at higher concentrations (Fig. 3A). Next, we examined whether increased concentrations of polyP (4-400 µg/Mouse ip) could enhance permeability in different organs in systemic short-term treatment. We observed that higher doses of polyP could increase vascular permeability in most organs including liver, lung, brain and heart (Fig. 3B-E). Although all animals survived during the experiment, but lethargy was noted in mice treated with polyP at higher doses (400 µg).

Next, we investigated the effect of long-term (12 days) systemic administration of polyP on vascular integrity in brain, lung, liver, and heart. In contrast to the potent pro-coagulant and pro-inflammatory functions of polyP, long-term administrations of lower doses of polyP (4 and 40 µg/Mouse) were well-tolerated. No significant complications were observed during long-term treatment at low polyP concentrations. Due to potential fatality at a high dose of polyP (400µg/Mouse) during systemic short-term treatment, long-term high dose treatment studies were not initiated. Results revealed that polyP significantly increased vascular permeability in all

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organs in a concentration-dependent manner (Fig. 3F-I). These results support the barrier disruptive effects of polyP in local, systemic short- and long-term treatment in different organs which highlight the clinical necessity of finding novel drugs in decreasing polyP-induced complications in inflammatory and coagulopathy diseases.

Metformin inhibits polyP-induced permeability in local and systemic short-term treatment

It has been shown that polyP-induced vascular leakage may partially be due to its activation of the mTOR signaling pathway [36]. Since the AMPK pathway inhibits mTOR signaling by activation of tuberous sclerosis complex [56] [57], we investigated the regulatory effects of metformin on polyP-induced hyper-permeability. Metformin is a well-known AMPK activator that is currently used for the treatment of type 2 diabetes mellitus [58]. Our results showed that metformin significantly attenuated polyP-enhanced hyper-permeability in skin (P<0.01) (Fig. 4A). The inhibitory effect of metformin on polyP-mediated vascular leakage was partially abrogated in the presence of dorsomorphin, an AMPK inhibitor, supporting the possible role of AMPK activation in the anti-inflammatory signaling function of metformin in polyP-stimulated mice.

Since during the systemic short-term treatment, administration of polyP at high concentrations (400 μg) was associated with lethargy, a lower concentration of polyP was chosen (40 μg/Mouse) to evaluate the protective effects of metformin on polyP-induced permeability in brain, lung, liver, ventricle, kidney, skin and spleen in this model. Consistent with the results of the local model, we showed that metformin can reduce the polyP-induced vascular permeability in the systemic short-term model as well (Fig. 4B-G). To further investigate the anti-inflammatory mechanisms of metformin we compared its protective effect in the presence and absence of dorsomorphin. Results showed that dorsomorphin decreased the protective role of metformin in polyP-induced hyper-permeability in different organs, suggesting the potential clinical value of metformin and possibly also other AMPK activators in attenuating polyP-associated vascular leakage and inflammation in different organs (Fig. 4B-G).

Metformin inhibits polyP-induced oxidative stress in systemic short-term treatment

To further investigate the anti-inflammatory mechanisms of metformin in preventing barrier disruptive effect of polyP, we measured the oxidant/anti-oxidant balance in different organs including brain, heart, kidney, lung and liver. We showed that polyP increased the level of malondialdehyde (MDA), an oxidative stress biomarker derived from lipid peroxidation, (Fig. 5A) while it reduced antioxidant markers including thiol concentrations (Fig. 5B), superoxide dismutase (SOD) (Fig. 5C), and catalase activities (Fig. 5D) in all harvested organs. These inflammatory properties of polyP were diminished in the presence of metformin, and dorsomorphin treatment decreased the protective effects of metformin on polyP-induced oxidative stress (Fig. 5A-D). Similar results were obtained when ROS generation was measured in endothelial cells using DCFDA cellular reactive oxygen species detection assay kit (Fig. 6A-B).

To further investigate the inhibitory effect of metformin on polyP-induced inflammation, Haematoxylin and Eosin (H&E) staining of tissues were evaluated in the systemic short-term model. Histological studies showed that polyP induced inflammatory cell infiltration in both organs with higher potency observed in lungs than skin. Additionally, significant edema and skin thickness (Fig. 6C), alveolar hemorrhage, lung edema (perivascular) and alveolar wall thickening (Fig. 6D) were other pathologies observed in the polyP-treated group. Metformin repressed polyP-induced infiltration of leukocytes and relatively normalize alveolar wall and structure in the absence but not the presence of dorsomorphin, possibly suggesting that metformin is exerting its protective effects through activation of the AMPK signaling pathway (Fig. 6C-D).

Discussion

We showed that polyP enhances vascular permeability in skin, liver, kidney, brain, heart, and lung in different inflammatory mice models. In this study, we showed for the first time that

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metformin can abrogate polyP-mediated inflammatory responses in cellular and animal models, possibly through activation of the AMPK signaling pathway.

The underlying mechanisms involved in vascular leakage are largly unknown. These mechanisms could be different between tissues and depend on the specialized vasculature including continuous, fenestrated, or sinusoidal. There are two main models for regulation of vascular permeability. One is the formation of transendothelial channels from vesicles or vacuoles, the vesiculo-vacuolar organelle (VVO), and the other involves endothelial junctions that can be transiently dissolved and allow extravasation. Peripheral tissues such as skin are composed of continuous and/or fenestrated blood vessels, which have relatively tighter interendothelial junctions between endothelial cells (paracellular permeability), compared to blood capillaries in the internal organs, especially tight junctions in blood-brain barrier endothelial cells. This process is complicated and further studies are required to refine our understanding of mechanisms regulating vascular permeability in different tissues [59].

It has been shown that metformin inhibits vascular inflammatory responses via suppression of NF-κB signaling as well as inhibition of the release of pro-inflammatory cytokines such as TNF-α, Interleukin (IL)-6, and IL-1β [60]. In a rat peritonitis model, metformin also diminished acute inflammatory responses via suppression of ROS accumulation, leukocyte migration and fluid extravasation [61]. Our results showed that metformin by decreasing concentrations of oxidant molecules like malondialdehyde and increasing activity or concentrations of anti-oxidant molecules including catalase and total thiol, respectively, regulates oxidant/anti-oxidant balance thereby suppressing polyP-induced inflammation. Barrier disruptive effect of polyP is the subsequent consequence of many proinflammatory responses in which oxidative stress is only one of them. Further studies should be performed to determine the potency of each of anti-inflammatory responses of metformin in suppressing polyP-induced hyper-permeability.

Furthermore, Zhao et al. demonstrated that metformin attenuates glioma-stimulated blood-brain barrier permeability and the expression of aquaporin (AQP)-4, thereby reducing cerebral edema *in vivo* [62]. Consistently, we demonstrated that pre-administration of metformin significantly attenuates hyper-permeability responses of polyP in local, systemic short- and long-term murine models. These results support the therapeutic potential of metformin as a FDA-approved drug in managing complications in polyP-mediated inflammatory diseases. The underlying molecular mechanisms of metformin remain controversial, but, it is generally agreed that this drug activates AMPK signaling pathway [63]. We also showed that metformin, at least partially, via activation of AMPK signaling pathway abrogates the disruptive effect of polyP in different organs. It is well-known that there is interplay between AMPK and mTOR signaling.

There are several studies supporting the role of mTOR signaling in regulating inflammation by increasing recruitment of inflammatory cells to injured tissues, releasing inflammatory cytokines , and increasing in vascular permeability [64, 65]. We previously showed that polyP regulates inflammation through up-regulation of the mTORC 1 and 2 signaling pathways in vascular the system [36]. More specificity, administration of siRNA against specific components of mTORC 1 and 2 abrogated polyP-induced hyper-permeability and NF-κB activation, respectively. Furthermore, it has been shown that mTOR signaling is repressed via the activation of AMPK [37] at least by two mechanisms including phosphorylation and activation of TSC2 [38] or directly through mTOR phosphorylation [39].

AMPK-dependent anti-inflammatory signaling functions of metformin have been well established. In line with this, Jian et al. showed a protective effect for metformin in reducing sepsis-induced acute pulmonary micro-vascular injury, vascular leakage, and barrier resistance and these effect were mediated through activation of AMPK in endothelial cells [66]. Similarly, metformin has been shown to suppress TNF-α-induced NF-κB up-regulation and attenuate the expression of cell adhesion molecules and inflammatory cytokines via activation of AMPK in HUVECs [43]. Furthermore, Park et al. demonstrated that the inhibitory effect of metformin

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against eosinophilic-mediated inflammation and remodeling is associated with AMPK activation in asthmatic mice [44]. These results are consistent with protective effects of metformin against endothelial cell function in diabetes patients [67, 68].

In summary, our findings support the significance of AMPK signaling pathway in the protective signaling functions of metformin against polyP-induced inflammation. We suggest further pre-clinical investigations to study the exact mechanism involved in protective role of this anti-inflammatory drug for better understanding and hence better management of polyP-associated inflammatory diseases.

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Conflict of interest

Authors have no conflict of interest.

Figure legends

Figure 1. Assessing the biological significance of proteins that interact with polyP. (A) Pathway analysis of proteins that are shown to interact with polyP. Significantly enriched pathways based on p-value < 0.05 are shown as canvas. The significant pathways after adjustment of p-value are shown in the adjacent Table. (B) Enrichment of biological processes of GO associated with polyP interacting proteins. The canvas shows processes with significant p-value <0.05. Significant processes after adjustments are shown in the adjacent Table.(C) PCA analysis on cell line expression profiles after treatment with metformin to select the most consensus alterations. (D) PolyP direct targets whose expressions are significantly affected by metformin. (E) Differentially expressed genes by metformin whose associated pathways overlap with those affected by polyP. WikiPathways and KEGG libraries were used for enrichment analysis.

Figure 2. Schematic representation of the experimental protocol

Figure 3. PolyP induces hyper-permeability in local and systemic short-and long-term models. (A) Administration of 50 µg/mouse polyP significantly increased permeability in the skin. However, higher doses represent lower vascular leakage in the local treatment (50-200 µg/Mouse id). An increase in polyP dosage increased in blood vessel permeability in (B) lung, (C) heart, (D) liver, and (E) brain tissues. The experiment on systemic long-term model provided same results in (F) lung, (G) heart, (H) liver, and (I) brain. *p<0.05; **P<0.01; ***P<0.001 (n=6 in each group).

Figure 4. Metformin decreased polyP-induced hyper-permeability in local and short-term systematic models. (A) Metformin significantly reduced the polyP-induced permeability

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whereas administration of dorsomorphin suppressed inhibitory effects of metformin on polyP-induced hyper-permeability in the local model. The protective effects of metformin on hyper-permeability effects of polyP (40 μg/Mouse) has been compared in (B) lung, (C) heart, (D) liver, (E) brain, (F) kidney, and (G) spleen in systematic short-term model. *p<0.05; **P<0.01; ***P<0.001 (n=6 in each group).

Figure 5. Metformin regulated polyP-induced oxidative stress in systemic short-term treatment. The concentration of malondialdehyde (MDA) (A) and total thiol (B), as well as superoxide dismutase (SOD) (C) , and catalase activities (D) were compared between groups in lung, heart, liver, brain, and kidney. *p<0.05; **P<0.01; ***P<0.001 (n=6 in each group).

Figure 6. Metformin attenuated polyP-induced ROS generation and inflammatory process in short-term systematic model. (A-B) Metformin and NAC reduce the polyP-induced ROS production in endothelial cells. **P<0.01 compared to saline group. (C-D) Hematoxylin and eosin (H&E) staining represented a significant presence of inflammatory cells infiltration, more edema (black arrows) and skin thickness (C) in polyP-treated mice. Similarly, alveolar hemorrhage (black arrows), lung edema (perivascular) (black dash type arrows) and alveolar wall thickening (arrow heads) were observed in (D) lung tissues in the polyP group. Metformin reduced the inflammation in the skin (C) and in the lung (D) in the absence but not the presence of dorsomorphin (n=6 in each group).
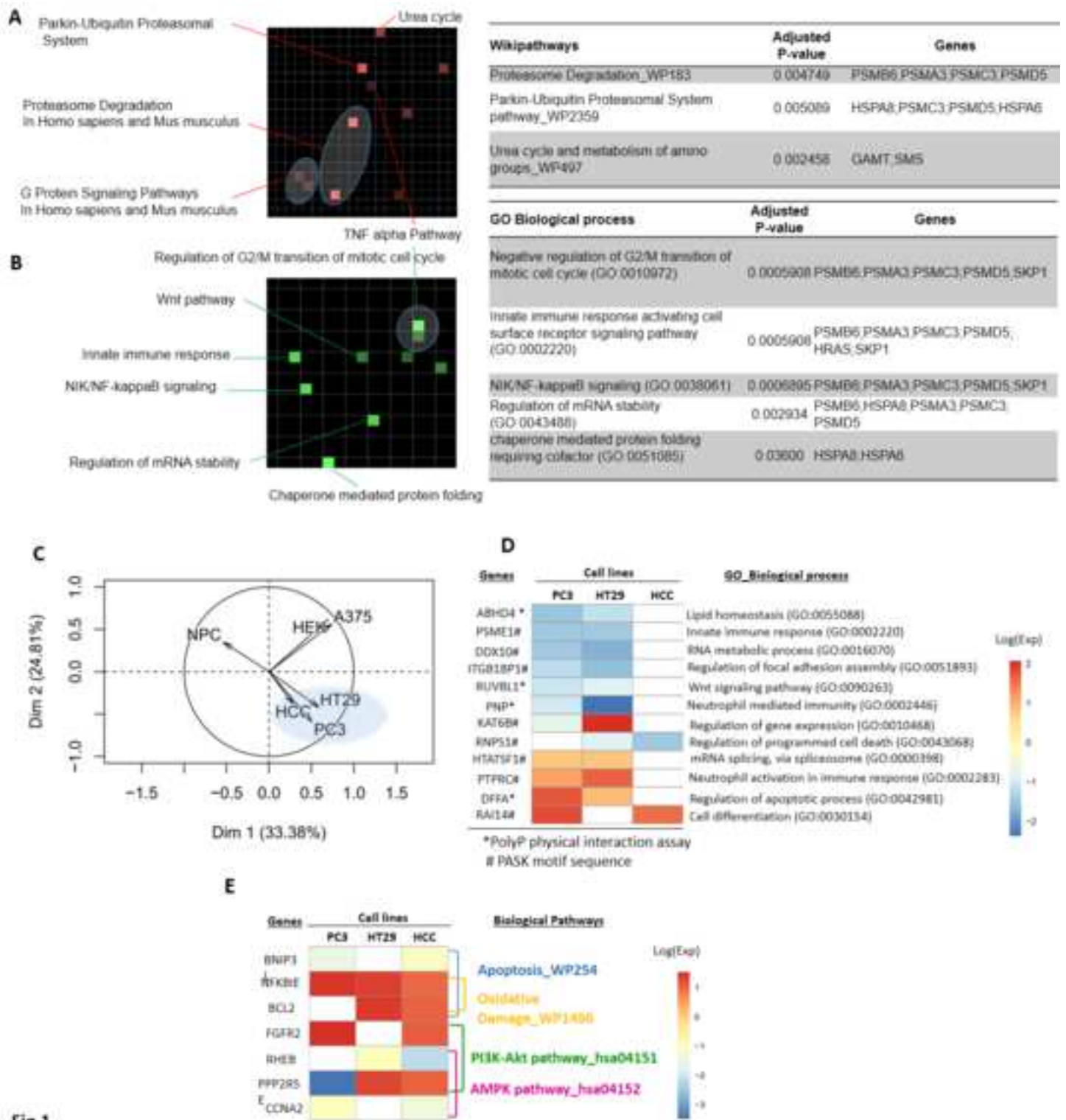
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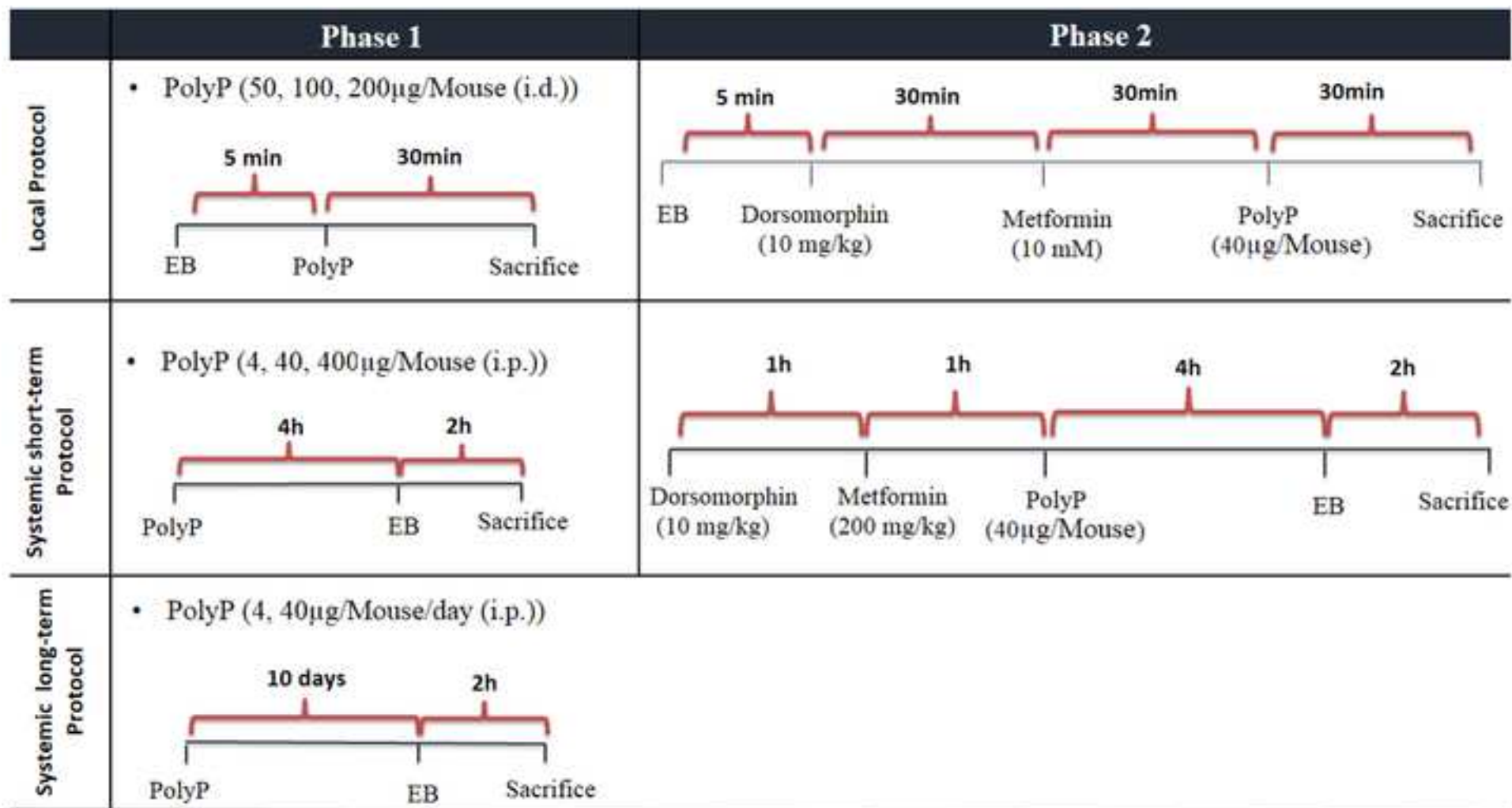
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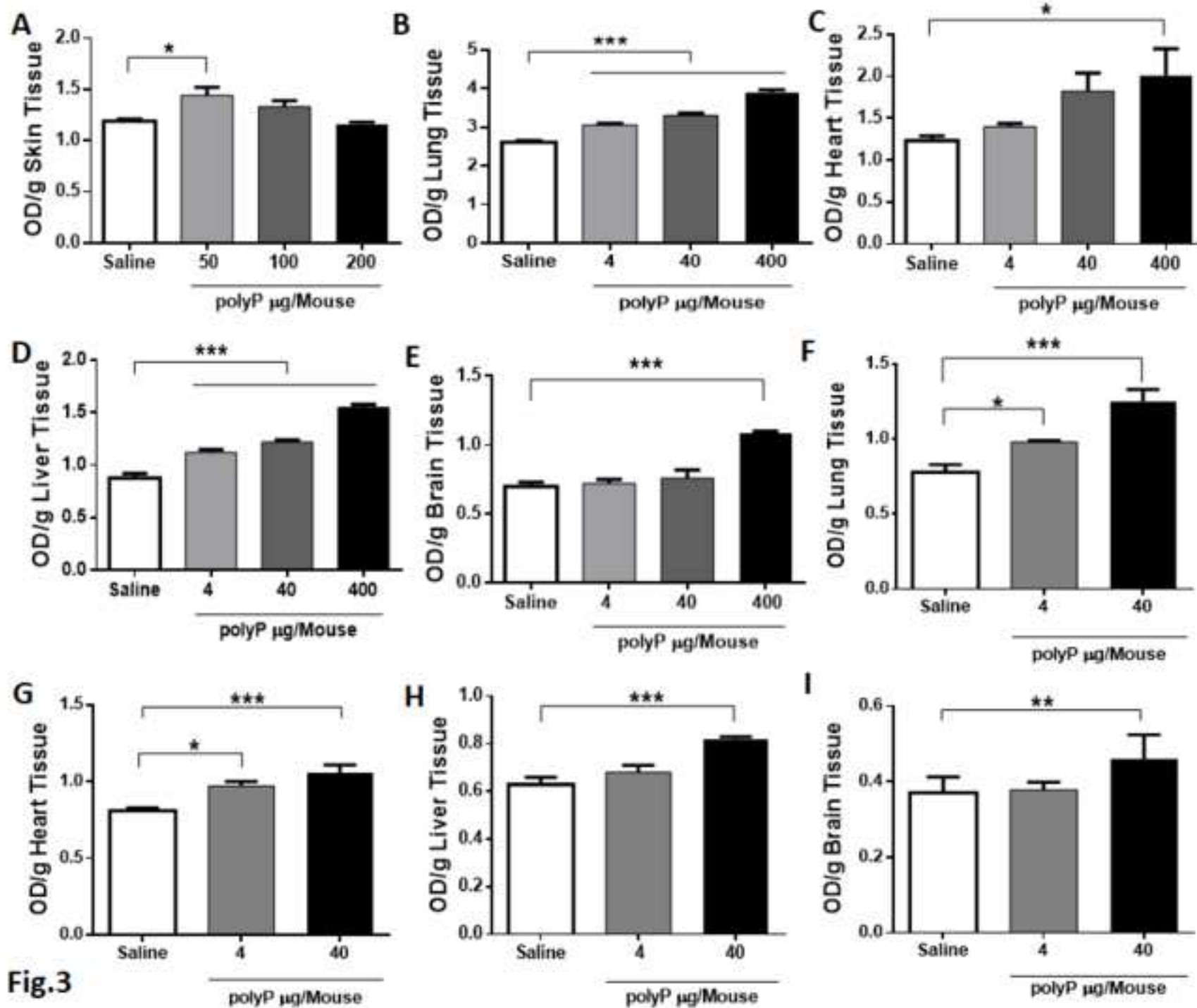
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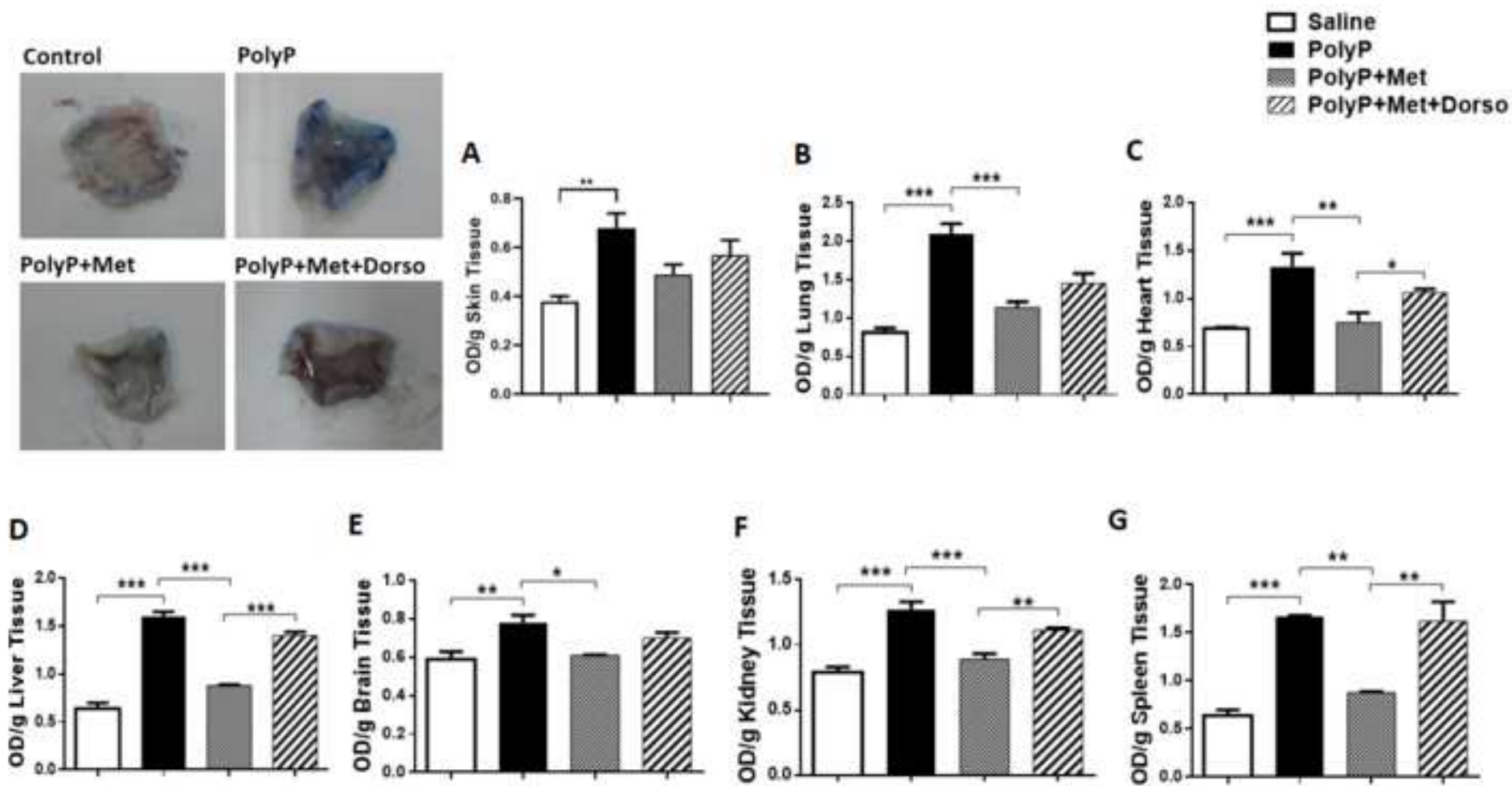
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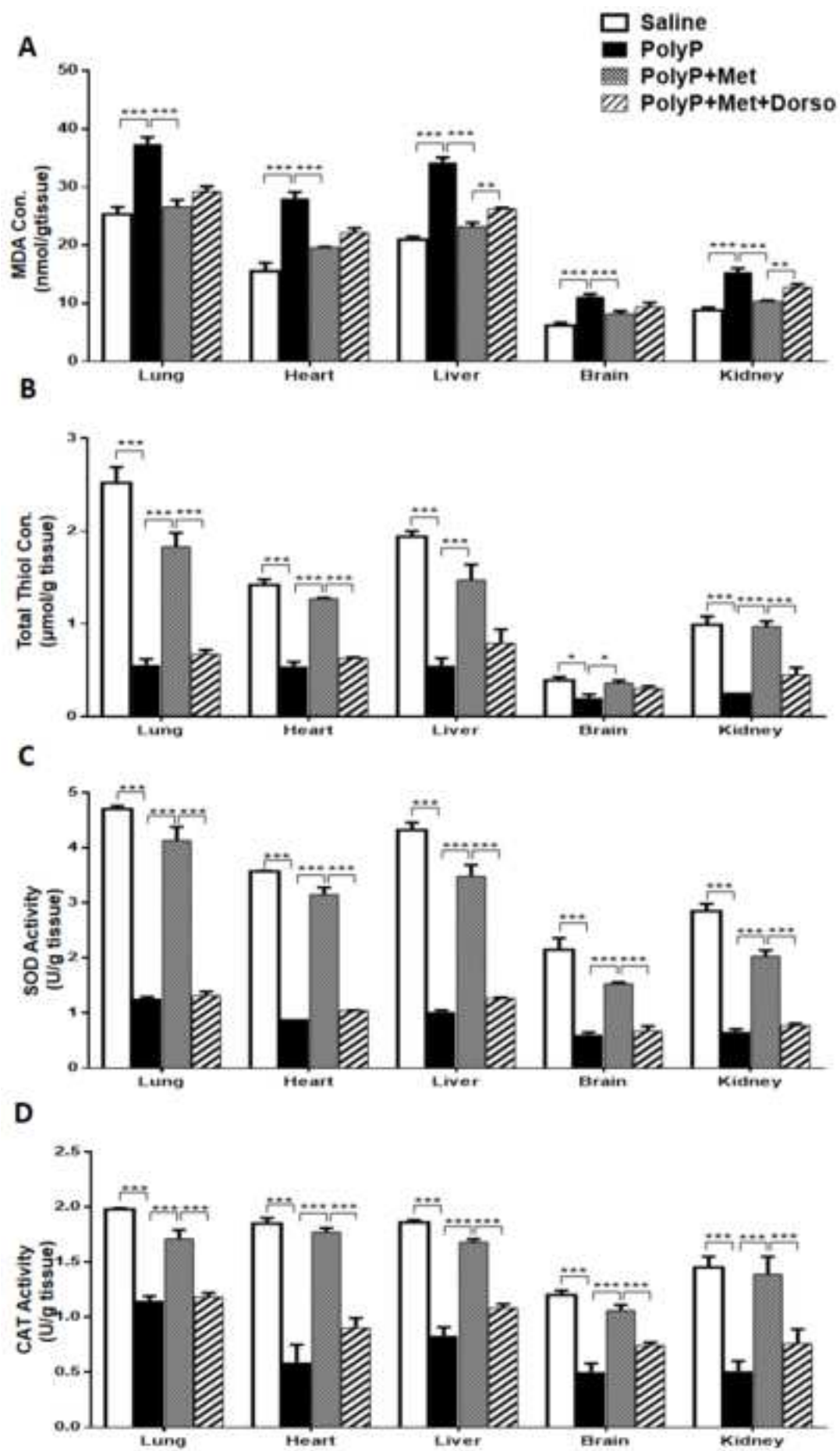
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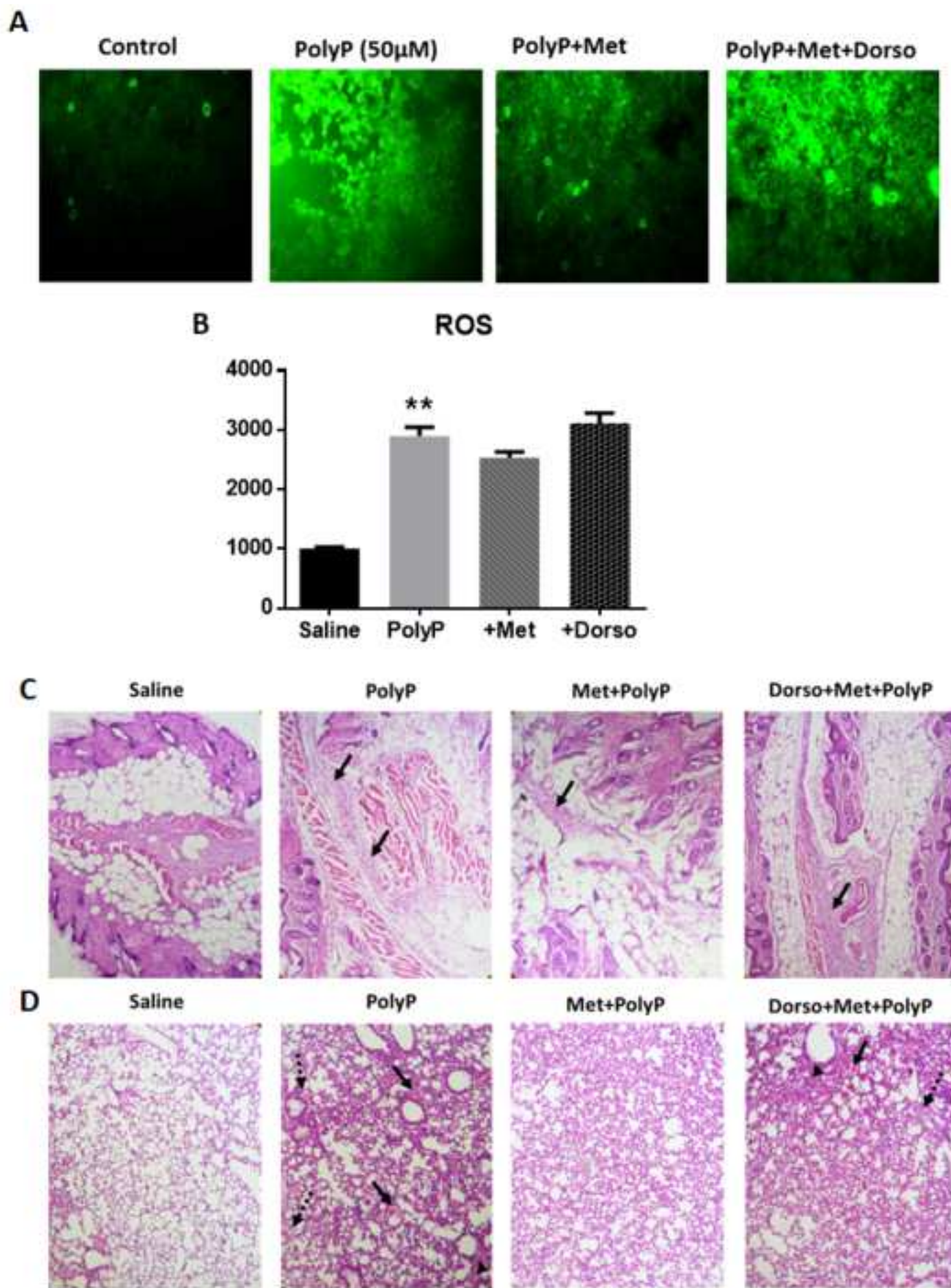
Fig.6

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Metformin inhibits polyphosphate-induced hyper-permeability and inflammation

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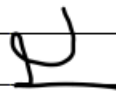
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Acknowledgements

All persons who have made substantial contributions to the work reported in the manuscript (e.g.,
technical
help, writing and editing assistance, general support), but who do not meet the criteria for authorship,
are
named in the Acknowledgements and have given us their written permission to be named. If we have
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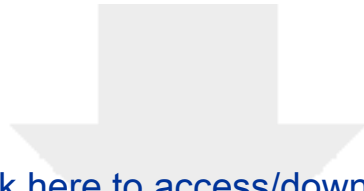
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