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Attenuation of internal organ damages by exogenously administered epidermal growth factor (EGF) in burned rodents

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Abstract

Major burns are associated with multiple internal organ damages, including necrosis of the gastrointestinal mucosa. Failure of the intestinal barrier is a serious complication in burned patients. Epidermal growth factor (EGF) is a mitogenic polypeptide that stimulates wound repair and affords protection to the gastric mucosa. We examined whether a single systemic intervention with EGF prevents organ systems damages, following full-thickness scalds (25–30%) in rodents. Animals were randomly assigned to receive an intraperitoneal injection of EGF (30 μg/kg in mice, 10 μg/kg in rats) or saline solution, 30 min prior thermal injury in mice or after the cutaneous injury in rats. General clinical condition and mortality during 24 h were recorded. Animals were autopsied and histopathological and histomorphometric studies were conducted. Mice treated with EGF exhibited a milder clinical evolution and acute lethality was significantly reduced as compared to saline counterparts ($P < 0.01$). Histopathological and morphometric analysis showed that EGF significantly reduced intestinal necrosis and contributed to preserve jejunoileal architecture in mice ($P < 0.05$) and rats ($P < 0.01$). The onset of renal hemorrhagic foci was significantly reduced in EGF-treated groups ($P < 0.01$). Lung damages appeared attenuated in EGF-treated animals. These data indicate the salutary effects of EGF by attenuating internal complications associated to thermal injuries. Further studies are warranted to fully elucidate the usefulness of this therapy.

Keywords: Epidermal growth factor (EGF); Burns; Cytoprotection

1. Introduction

Severe cutaneous thermal traumas are associated with multiple organ dysfunction, which demand urgent and multidisciplinary medical interventions to ensure patient's survival [1].

Clinical data indicate that gut blood flow remains open to pharmacologic interventions in order to attenuate splanchnic circulatory impairments even when global indices of tissue perfusion are adequate [2].

Gut hypoperfusion leads to structural mucosal damages and to subsequent barrier failure [3]. Loss of the intestinal barrier function is a life-threatening complication that allows bacteria and their toxins to enter the systemic circulation. Gut-derived bacteremia and/or endotoxemia are potent signals that trigger or exacerbate the hypermetabolic and immune inflammatory responses. Furthermore, the increased production of cytokines by the "stressed" intestine is considered a critical component in MOF pathophysiology [3]. These facts suggest that preservation of the intestinal mucosal barrier is, therefore, of paramount clinical importance for the outcome of burn victims.

Epidermal growth factor (EGF) is a mitogenic peptide that is secreted by salivary and duodenal Brunner's glands; it is the most extensively studied of all the peptides involved in gastrointestinal mucosal integrity. In addition of enhancing epithelial repair, EGF is a potent cytoprotective and trophic agent for the gastrointestinal epithelium [4].

Although a variety of experiments clearly illustrate that exogenously administered EGF protects the gastrointestinal mucosa against the noxious effects of different ulcerogenic factors and stressful events [5], the potential benefits of this polypeptide in preventing acute post-burn splanchnic organ damages are scarce. To our knowledge, the only previous report in this regard was provided by Zapata-Sirvent et al. [6] who showed that repeated EGF interventions following scalding, reduces bacterial translocation by attenuating gastrointestinal mucosal necrosis.

Here, we examined the effects of the parenterally administered EGF either before or after exposing animals to a thermal insult. Our results show that a single intervention with EGF is able to confer gastrointestinal protection, with consistent preservation of the mucosal viability and architectural

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integrity. Furthermore, EGF administration contributed to attenuate renal and lung parenchymal damages. Pre-treatment with EGF significantly enhanced survival of burned mice. These data support and extends previous findings that encourage further experiments to fully elucidate the relevance of the systemic EGF therapy in major burn victims.

2. Materials and methods

2.1. Animals

Male Balb/c mice (22–25 g) and Sprague Dawley male rats (250–270 g) were purchased from the National Center for Laboratory Animals (CENPALAB, Havana, Cuba). Animals were individually caged under certified and controlled environmental conditions and were acclimated for 7 days before the experiment. Food and water were not restricted so that early feeding was allowed for all the animals following the thermal trauma.

2.2. Ethics

All the experiments were performed according to current regulatory guidelines of the Animal Welfare Committee of the Center for Genetic Engineering and Biotechnology, Havana, Cuba. Appropriate sedation and anesthesia were used for all the painful procedures (see below).

2.3. EGF

Commercially available recombinant human EGF_{1-52} was obtained from Heber-Biotec SA, Havana, Cuba. EGF protein concentration was determined by an ELISA system [7] and biological activity was measured by thymidine incorporation in primary cultured hepatocytes [8]. EGF was dissolved in sterile 0.9% saline solution and filter (0.22 μm; Minisart, Sartorius, Germany) sterilized before administration.

2.4. Burn injury

Animals received diazepam (40 mg/kg) and atropine (0.02 mg/kg) as pre-anesthetic medication [9]. Deep anesthesia was induced with ketamine (40 mg/kg) accompanied by a brief exposure to diethylether mask before scalding. Solutions were intraperitoneally administered. Dorsal scald injuries were reproduced as described by Walker and Mason [10]. Once the dorsum of the animals was depilated, pre-delimited areas were immersed in equilibrated water at 98–99 °C for 10 and 5 s in rats and mice, respectively; resulting in approximately a 25–30% of burned body surface area for both species [11]. Total burned body surface area calculations were done as referred [12]. All the animals were immediately resuscitated after scalding with 0.9% normal saline solution (50 ml/kg) via intraperitoneal injection [13] Injury depth was histologically confirmed as full-thickness burn wounds 8 h following induction.

2.5. Experimental protocols

Three independent experiments with mice were conducted including a total of 20 mice in each series. For each study, 10 mice were randomly assigned to treatment with EGF at 30 μg/kg or to receive saline solution. Both solutions were intraperitoneally administered 30 min before thermal injury infliction. In the experiment with rats, a total of 14 rats received thermal injuries. Seven animals were randomly assigned to treatment with EGF at 10 μg/kg or saline solution administered intraperitoneally, 10 min after the injury. EGF doses used here are the minimal doses conferring protection along pilot experiments using both species. No further EGF administrations were conducted in either mice or rats. Animals were frequently inspected and relevant clinical symptoms and lethality were recorded. Animals were sacrificed 24 h post-injury by ketamine overdose. Moribund animals were monitored until death to collect useful tissue samples. All the organs were inspected at autopsy and relevant information collected. The stomachs were opened, extended mucosal side-up and rinsed with saline and 10% buffered formalin. Gross mucosal damages were assessed under a dissecting microscope and representative fragments were harvested from damaged or otherwise normal-appearing mucosa. Intestines were similarly manipulated. The small and large intestines were thoroughly dissected, inspected and strips from duodenum, jejunum, ileum and colon were carefully extended and fixed in situ with formalin. Samples from the gastrointestinal tract, heart, lungs, spleen and pancreas were paraffin processed, sectioned at 5 μm and stained with hematoxylin and eosin for histopathological examination. Both kidneys from each animal were pole-to-pole dissected and hemisections processed to quantify the total number of hemorrhagic foci in the parenchyma of the kidneys using a magnification of 10 and 20×.

2.6. Morphometric procedures

In order to confirm the actual size of the burns, the injured areas in mice and rats were traced onto transparent plastic sheets and then measured using the DIGIPAT image-processing software. Intestinal morphometric analyses were performed on four to five fragments (≈2 CM each) collected from jejunum and ileum from each animal. Jejunal villi morphology was studied to assess changes in width, height and shape of each individual villous. The extent of damaged intestinal mucosa (DIM) was also measured. To achieve consistent data along morphometric studies, consecutive microscopic fields were examined at uniform intervals from each intestinal specimen. Microscopic images were captured with a Sony DXC-107 video camera coupled to a microscope (Leitz, Germany) at a constant 4× magnification and recorded in an IBM computer using a video

blaster SE-100 frame-grabber card. Each microscopic field extended for approximately 1500–1600 μm and included about 15–20 villi in the case of rats and 25–30 villi for mice. Villi were counted on each microscopic field and their base width and height measured. To quantitatively express changes in villous shape, a shape factor (SF) was introduced. The SF was calculated as:

$$\mathrm{SF} = \frac{AH}{W},$$

where A is the measured villous area, W the width of each villous and H the height of the villous (all expressed in μm). The SF varied from 0 to 1. Normal villi, which exhibited the typical digit-like shape, had a SF of approximately 1. In contrast, damaged villi, which exhibited a flatter appearance with a wider base and contracted height, generated a SF below or close to 0.5.

The DIM was calculated by measuring the length of intestinal mucosa showing necrotic denudation. It was expressed as the percent of total intestinal mucosal length for each microscopic field. All measurements were carried out using the DIGIPAT morphometric image-processing package (EICISOFT, Havana, Cuba). The average and standard deviation (S.D.) of all the measured parameters were calculated for each animal using the Microsoft Excel spreadsheet program. All the qualitative and quantitative determinations described here were blindly conducted using a sham code created by an external investigator.

2.7. Statistical methods

A two-tailed t-test was used to compare the size of the burns and the corresponding percentage of burned area in EGF-treated and saline controls in both species. The χ^2-test was used to compare cumulative post-burn lethality data between the two experimental groups in mice. The number of renal hemorrhagic foci in both experimental groups in mice and rats was evaluated for statistical difference using the non-parametric Mann–Whitney U-test. The morphometric parameters (namely villous height, villous base width, villous SF and the length of DIM) were evaluated for normal distribution on the two experimental groups in mice and rats using the Kolmogorov–Smirnov test ($P < 0.05$). When a normal distribution was found, the Student's t-test was used for means comparison. Otherwise, the non-parametric Mann–Whitney U-test was used. A value of $P \leq 0.05$ was considered as significant.

3. Results

3.1. Burn model

No statistical differences were detected in relation to the percentages of burned body surface area between EGF-treated and saline control animals (rats, $P = 0.75$; mice, $P = 0.90$). Histopathological examination of the scalded skin revealed that injuries were similar in terms of depth for either control or EGF-treated animals. No animals were excluded by erroneous scalding procedures.

3.2. Post-burn lethality and clinical disorders

By 6 h post-injury, control mice receiving saline were lethargic and exhibited mild to severe neurological depression as judged by their poor or null responsiveness to environmental stimuli. This neurological impairment was transient in most animals and normal behavior was recovered generally about 10–12 h post-injury. Other clinical complications, such as hematuria, abdominal distension and diarrhea were observed in mice receiving saline. The characteristic clinical picture of moribund animals also included marked hypothermia, respiratory depression and unresponsiveness to stimuli. The onset of these complications ranged between 12 and 16 h after the scalding. Mice pre-treated with EGF exhibited only mild prostration and their clinical appearance improved along the time. Hematuria, diarrhea and the abdominal dilation were completely prevented by the EGF pre-treatment. Cumulative data from the three independent experiments demonstrated that survival was significantly higher in the groups of mice pre-treated with EGF (Table 1).

Rats, in general, were more tolerant than mice to the thermal trauma. Lethargy or unresponsiveness was not observed in either EGF- or saline-treated rats. Hematuria, abdominal distension, diarrhea, hypothermia, respiratory depression or acute lethality was not observed in rats.

3.3. Gross gastrointestinal changes

At necropsy, the gastrointestinal tract appeared to be the most affected tissue in both rats and mice. Gastric ulcers with hemorrhagic bottom or mucosal superficial erosions appeared in all the saline control rats. Gastric damages in mice were much milder and mostly associated with congestion or hemorrhage (Table 2). Undigested blood was observed in the lumen of the jejunal segment in both saline control mice and rats. Fibrin casts were also seen, which occupied the duodenum and upper jejunum lumens. No evidence of large bowel damage was observed in any of the species.

Table 1
Post-burn acute lethality in mice

Assay	EGF	Saline
1	0 (0%)	4 (40%)
2	2 (20%)	5 (50%)
3	0 (0%)	4 (40%)
Total	2 (6.6%)	13 (43%)

Lethality was registered during three independent experiments with 20 mice each (10:10). Comparison of the cumulative data (30 mice is 100%) showed that EGF treatment significantly reduced acute mortality in burned mice ($P = 0.0028$, χ^2-test).

Table 2
Number of animals per group with gross gastrointestinal injuries

Autopsy finding	EGF		Saline controls	
	Rats	Mice	Rats	Mice
Gastric ulcers	0	3	5	21
Gastric mucosal hemorrhage	0	0	4	19
Superficial erosions	1	1	7	16
Small intestine bleeding	0	0	6	24
Small intestine fibrin casts	0	2	7	26

EGF administration reduced or prevented gross gastrointestinal injuries in both mice and rats. No statistical comparisons were done due to obvious differences. Data shown for mice correspond to cumulative autopsy findings of three different experiments involving a total of 30 animals for either EGF or saline treatments. Fourteen rats were used, seven receiving EGF and the others saline solution.

As shown in Table 2, EGF treatment consistently afforded a protective effect on the gastrointestinal system in both species. Only two rats had superficial gastric erosions with no evidence of deep gastric ulcer or intestinal bleeding. Intestinal bleeding and fibrin deposits were also prevented in EGF-treated mice. During autopsy, abundant alimentary content appeared in 86 and 93% of the EGF-treated rats and mice, respectively, whereas for both species scant gastric content appeared in <30% of the saline control animals.

3.4. Histopathological examination: saline control animals

Histopathological examination confirmed that the gastrointestinal tissue was seriously damaged in both animal species by the thermal stress. Deep gastric ulcers and mucosal vascular congestion were confirmed. The most affected portions of the small intestine were jejunum and ileum. Different patterns of villi damages were observed in most of the segments studied and ranged from villi tips erosion to complete desquamation of the necrotic tissue with luminal hemorrhage (Fig. 1A). Other villi appeared flattened, coalescent and thickened in their base with mild infiltration of round cells, congestion and hemorrhage in the lamina propria. Some villi showed an atrophic aspect due to marked hypocellularity and lamina propria exhaustion. The inflammatory infiltrate was not intense even in areas of deep mucosal necrosis.

3.5. Histopathological examination: EGF-treated animals

Superficial desquamation of the gastric mucosa involving the epithelium and the upper region of the glands was observed particularly in rats. Ulcers were not detected, however, EGF-treated mice showed a relatively normal gastric mucosa. The intestinal mucosa looked well preserved. Most villi were relatively intact in all the specimens studied with only minimal epithelial erosion at the tip, associated with light edema and mild hypercellularity of the lamina propria. Deep intestinal necrosis was detected only as small patches along the jejunum or ileum. No evidence of extensive luminal hemorrhage was observed (Fig. 1B).

3.6. EGF treatment prevented renal parenchyma hemorrhage

Scattered foci of tubular epithelial necrosis were observed in all the burned animals. EGF treatment, either before or after the thermal insult, did not appear to prevent this process. In addition, tubular dilation and glomerular changes associated with congestion, hypercellularity and edema were found in both EGF- and saline-treated animals. Scattered hemorrhagic foci were frequently found in the medulla interstitium of control mice and rats. EGF treatment, significantly reduced the number of renal hemorrhagic foci more that two-fold in mice and four-fold in rats in relation to saline counterparts (Fig. 2).

3.7. Histopathological changes in other organs

Only minimal erosion was detected in the colonic mucosa in those animals with large stretches of jejunal or ileal mucosal denudation. A common finding in both EGF-treated and control groups was the marked vascular congestion in most of the organs studied. Hemorrhagic splenitis was relatively attenuated in the EGF-treated mice as compared to controls. Spleen specimens from dead mice exhibited evidences of intense intra-splenic haemolysis. No qualitative differences were observed in spleen histology in either EGF-treated or control rats. Leukostasis was observed in the microvasculature of different tissues of mice and rats (lungs, pancreas, heart, etc.). However, in qualitative terms, it seemed to be consistently reduced in animals that received EGF. Irrespective to the treatment received, the livers from mice and rats exhibited hydropic degeneration and hypertrophy of Kupffer's cells.

Differences in lung histopathology were detected among mice that had undergone a prolonged critical condition and that eventually succumbed as compared to those with milder evolution. Most importantly, clear qualitative differences were found in lungs from EGF pre-treated mice as compared to those receiving saline solution. In the latter, lungs exhibited larger foci of intense parenchymal collapse, alveolar walls were thicker with a remarkable hypercellularity that included inflammatory cells. In contrast, most of these changes appeared very attenuated in mice receiving EGF.

3.8. Morphometric assessment

As shown in Table 3, mice pre-treated with EGF had higher villi than the saline controls (365 ± 95 versus 288 ± 95, $P = 0.05$). Significant differences between the two groups were also detected in villi SF, indicating that villi shape in the EGF-treated mice was less disrupted.

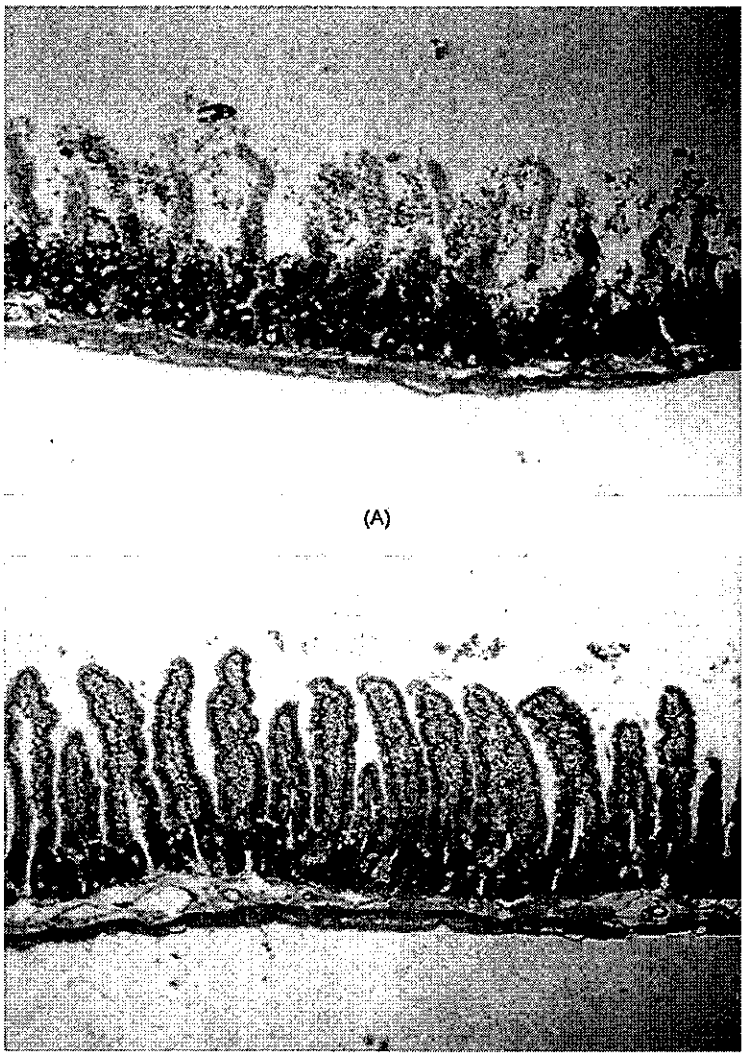

Fig. 1. (A) Representative image of the DIM detected in placebo-treated animals. Villi damage involved complete epithelial necrosis with desquamation and hemorrhage in lamina propria. Most of the crypts were relatively well preserved. (B) Representative aspect of the intestinal mucosa in animals receiving EGF therapy. The dramatic protection afforded by EGF is supported by the preservation of the different mucosal structures (5 μm section; H/E staining; magnification 10×).

Most importantly, pre-treatment with EGF provided significant mucosal protection as indicated by a 15% reduction of DIM in the EGF-treated compared to controls (25 ± 16 versus 9 ± 6, $P = 0.02$). Table 3 also shows that post-burn EGF treatment of rats preserved villous height as compared to saline controls (538 ± 88 versus 322 ± 48, $P = 0.001$). Villi of saline-treated rats also had wider bases and their morphology was significantly more disrupted than villi from EGF-treated rats; as indicated by the differences in SF between the two groups (0.76 ± 0.08 versus 0.94 ± 0.07, $P = 0.001$). Animals receiving saline solution also exhibited significantly more denuded intestinal mucosal area (DIM of $52 \pm 24\%$) as compared to the EGF-treated group (DIM of $11 \pm 5\%$) ($P = 0.001$).

4. Discussion

Despite the progress achieved in fluid resuscitation therapy to maintain an adequate cardiac output, burn victims still suffer splanchnic organs complications, such as acute stress gastric ulceration and intestinal mucosal necrosis [1,14]. Gut mucosal damages may be followed by bacterial and/or toxins translocation [15]. Therefore, in correspondence with the growing understanding of the molecular pathophysiology of the splanchnic microvascular failure, novel adjunctive therapies are required to attenuate acute intestinal and other post-burn internal damages.

The fact that a single prophylactic intervention with EGF activates cellular survival mechanisms, against agents that

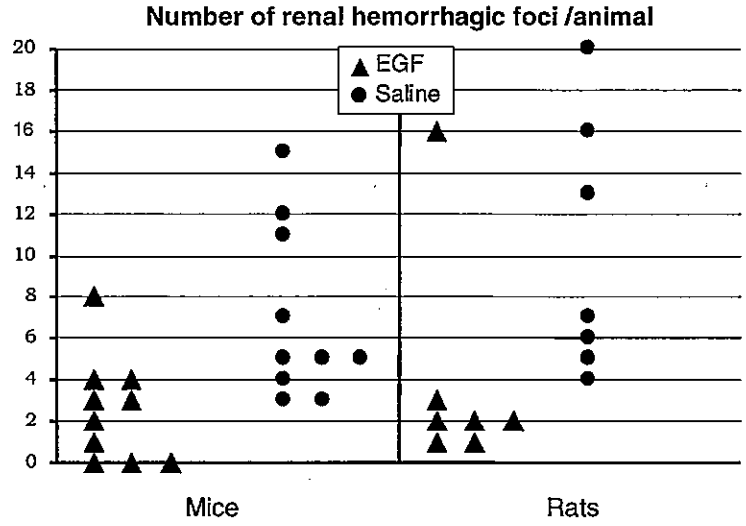

Fig. 2. EGF significantly reduced the number of renal interstitial hemorrhagic foci in rats ($P = 0.03$) and mice ($P = 0.009$; Mann–Whitney U-test). $P < 0.05$ was established as a significant value.

act through multiple pathophysiologic injury cascades, reflects the polyvalence of the EGF-mediated cytoprotection [16,17]. Current information is limited, however, in relation to the potential benefits of the EGF therapy as a cytoprotective factor in order to attenuate acute multiple internal organ damages following major burns. In this experiment, a single EGF bolus was either prophylactic or therapeutically administered. For the first one, mice were pre-treated 30 min before scalding and in the second, rats received EGF 10 min after the scald infliction. The prophylactic strategy was chosen to determine whether the EGF "pre-conditioning" would steadily persist following the subsequent thermal stress. The later strategy is more realistic for therapeutic goals. Results from the prophylactic intervention regimen indicated that a single EGF bolus was sufficient to induce multi-organ protection, suggesting that cellular pre-conditioning of key internal epithelial organs was achieved and that it was not aborted by the systemic disturbances associated to the subsequent thermal insult. The EGF therapeutic intervention regimen also proved to reduce internal organs damages, although rats clinical evolution was milder and lethality was not detected irrespective to the treatment applied. In general, our data indicate the beneficial effects of an EGF intervention by attenuating the severity of the post-burn clinical evolution and by reducing acute gastrointestinal, renal and lung morphological damages. In mice, EGF pre-treatment also significantly enhanced survival.

Table 3
Morphometric assessment of the intestinal damage

Villi height (μm)	Villi width (μm)	SF (AU)	DIM (%)
Mice treated with saline solution			
288 ± 95	85 ± 13	0.81 ± 0.09	25 ± 16
Mice treated with EGF			
365 ± 75*	77 ± 14	0.94 ± 0.08**	9 ± 6**
Rats treated with saline solution			
322 ± 48	130 ± 12	0.76 ± 0.08	52 ± 24
Rats treated with EGF			
538 ± 88*	95 ± 23*	0.94 ± 0.07*	11 ± 5*

Data are expressed as mean±S.D. SF is expressed in arbitrary units (AU). Animals treated with EGF exhibited a significant preservation in most of the intestinal mucosa architectural parameters studied. EGF intervention significantly reduced the extent of mucosal necrosis and denudation, expressed as DIM. Comparisons were done by Mann–Whitney U-test. Significance established for $P < 0.05$.

* $P < 0.01$.

** $P < 0.05$.

The severe disruption of the intestinal mucosa integrity was the most impressive consequence of the thermal insult in both rats and mice. The intestinal damage was characterized by necrosis of the villi accompanied by partial or complete epithelial desquamation, which progressed to mucosal necrosis with areas of denudation and luminal hemorrhage. All these intestinal mucosal changes appeared irrespective of having introduced an acute fluid resuscitation therapy [13] and despite allowing early feeding for all the animals. The relationship between a severe thermal injury and the failure of intestinal perfusion during early and late post-resuscitation periods has been demonstrated in a robust animal model [18] and in clinical studies [2]. Thus, our findings support the concept that shock resuscitation may restore global oxygen transport but not restore oxygen delivery neither its utilization due to splanchnic vasoconstriction [19]. Concomitantly, the severity of intestinal mucosal injury depends on the duration and intensity of the splanchnic hypoperfusion episode [3,20]. These data indicate that there may be two major adjunctive alternatives to preserve gut morphological and functional integrity: (i) acute restoration

of the splanchnic microvascular homeostasis; (ii) administration of pro-survival/cellular rescue agents.

EGF provided a significant protective effect on the intestinal mucosa, characterized by the substantial preservation of the native mucosal architecture, confirmed by histopathological and morphometric assessments. Although the functional relevance of this mucosal preservation in terms of mechanical barrier against bacterial and/or endotoxin translocation was not determined, it is conceivable that the EGF-treated animals were better protected against noxious translocation. Others have indicated that the extent of the intestinal mucosal injury correlates with the degree of gut-borne pathogens and/or toxins translocation [3]. The fact that stomachs of EGF-treated animals contained much more food than the saline counterparts suggests that EGF medication improved animals' general condition. The larger food consumption could have positively influenced on the gastrointestinal mucosa preservation as well.

The histopathological picture characterizing the intestinal lesions in our experiments correspond to descriptions of small bowel damages caused by experimental [21] and clinical [22] ischemia/reperfusion events. In all these processes, the common factor is a decrease in the splanchnic perfusion resulting in a concomitant reduction in effective oxygen delivery to the intestinal mucosa and uptake by the local cells. Superoxide radicals and other oxygen reactive species are produced which may cause mucosal injury by lipid peroxidation and by damaging cell membranes system and the mitochondria. At this point, this biochemical derangement is irreversible and lethal [23]. Other mediators/events, such as thromboxane release [24], nitric oxide over-production [25], complement activation [26] and burn wound-derived pro-inflammatory and cytotoxic agents [27] are involved in gut tissue injury and barrier failure pathogenesis.

EGF treatment completely prevented the appearance of acute gastric ulcers in burned animals. Ischemia, leading to luminal hyperacidity is invoked as a critical pathophysiologic factor for the onset of stress gastric ulcerations in extensively burned patients [28]. These data lead us to speculate that the cytoprotective role exerted by EGF on the gastrointestinal mucosa of the burned animals may be related with the prevention of local ischemia and/or with the protection of the cells against the consequences of ischemia/reperfusion, such as reactive oxygen species generation. The former hypothesis is supported by previous observations indicating that exogenously administered EGF stimulated blood flow to the gastrointestinal mucosa and enhanced splanchnic circulation in different animal species [29,30]. Other compelling evidence supports a putative anti-lipoperoxidant effect for EGF. Systemically administered EGF reduced lipid peroxidation in the gastric mucosa of cold-restrain stressed rats [31] and during a gastric ischemia/reperfusion episode [32]. Studies examining the effect of topically administering EGF to skin-burn sites [33] and to rat fetal lung cells stressed by hyperoxia [34] indicated that EGF stimulates the production of superoxide dismutase. Other evidences suggested that systemically administered EGF acted as a hepatoprotective agent by attenuating lipid peroxidation in rats exposed to a chemical hepatotoxin [35]. Thus, speculatively, EGF may act by a dual mechanism to reduce gastrointestinal damages following a major burn trauma; by reducing intestinal hypoperfusion and/or by attenuating ischemia/reperfusion-derived damages. Other findings that argue in support of the EGF mediated multi-organ protection are the prevention of renal parenchymal hemorrhage, the attenuation of neutrophilic leukostasis in a variety of organs and the preservation of the lung parenchymal morphology. The fact that EGF did not modify the incidence of acute tubular necrosis in burned animals may be related to a matter of dose sizes, rather than the inefficiency of the growth factor itself to confer global nephroprotection against ischemia. Two independent studies of our group have demonstrated that a single prophylactic or therapeutic administration of EGF prevents renal morphological, functional and biochemical deterioration in a chemical model of multiple organ failure [36] and following an ischemia/reperfusion episode [37]. In both studies, EGF effects appeared in a dose dependent manner, being relevant at 750 μg/kg. Further experiments are envisioned to fully elucidate this issue.

The attenuation of neutrophilic/endothelial adhesion and recruitment in multiple organs, including the lungs in EGF-treated animals, may be interpreted as a signal of protection against tissue hipoxia, low flow states and/or against the over-production of reactive oxygen species. These events are interconnected, and are known to mediate the over-expression of cell adhesion molecules and to amplify tissue damages [15].

EGF proved to be a potent stabilizer of the gastrointestinal epithelial population homeostasis, as showed to ameliorate the morphological deterioration of central organs involved in MOF onset. Introducing EGF in human clinical conditions, such as severe burns that urgently demand the preservation of the intestinal barrier may contribute to reduce morbidity and mortality.

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