SOME CONSIDERATIONS ON THE PHYSIOLOGICAL ROLE OF EPIDERMAL GROWTH FACTOR IN RELATION TO ITS PHARMACOLOGICAL APPLICATIONS

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ABSTRACT

Epidermal growth factor (EGF) is a polypeptide growth factor, which stimulates cellular division, migration and differentiation. A number of *in vivo* models have extensively documented that EGF is involved in embryonic, fetal and neonatal development, differentiation and maturation. Other evidences clearly show that EGF is a fundamental growth factor in maintaining tissue morphology and physiology in adult organisms, whereas it seems to participate in cytoprotection, cellular population renewal and in epithelial healing. Some data suggest that the parenteral administration of EGF in pharmacological concentrations provokes effects that may be significantly beneficial to prevent or treat visceral organic pathologies. As preclinical results conducted so far suggest that EGF does not initiate malignant transformation, its parenteral use for acute disorders or life-threatening conditions is likely to be useful on the basis of an individual benefit-risk ratio in patients.

Key words: EGF, physiology, pharmacology, parenteral

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RESUMEN

El factor de crecimiento epidérmico (FCE) estimula eventos mitóticos, motogénicos y diferenciativos en células que expresan su receptor. Numerosos datos ilustran la participación del FCE en el desarrollo embrionario, fetal y la maduración neonatal. En organismos adultos el FCE parece estar involucrado en el recambio de poblaciones epiteliales, en la citoprotección y en la cicatrización de algunos tejidos ulcerados. La administración parenteral de FCE en concentraciones farmacológicas, provoca significativas respuestas tisulares que pueden ser más extensamente explotadas en el orden terapéutico, para prevenir o tratar daños morfofuncionales internos. El hecho de que las pruebas preclínicas efectuadas hasta ahora, muestren que el FCE no inicia transformación maligna, argumenta la posibilidad de emplear este polipéptido por vía parenteral, en patologías de curso agudo o de pronóstico crítico sobre la base del análisis del radio riesgo-beneficio en cada paciente.

Palabras claves: FCE, fisiología, farmacología, parenteral

Introduction

Epidermal growth factor (EGF) was originally isolated from the mouse submandibular glands as a concomitant of nerve growth factor and since then, it has been recognized by its ability to stimulate precocious incisor eruption and eyelid opening in newborn mice (1).

Substantial amounts of immunoreactive EGF are found in a variety of human tissue extracts and body fluids as amniotic fluid, milk, saliva, gastric and duodenal content, pancreatic juice, bile, urine (2-7), whole blood, serum and platelet-poor plasma (8, 9). This led to suggest that this polypeptide may act in an autocrine, paracrine, or more remotely in an endocrine fashion (10).

Although EGF was discovered more than thirty years ago, its definitive physiological role in intact organism is under current investigation (11). Nevertheless, EGF seems to be a fundamental polypeptide growth factor in cellular differentiation, development, and tissue protection and repair (10).

Soon after EGF was isolated and its biological effects on peripheral epithelial tissues were characterized, a plethora of *in vivo* models appeared demonstrating that salivary EGF enhanced skin wound healing (12). However, there is current consensus in that some pre-requisites should be met for EGF to significantly enhance skin wound healing, i.e., prolonged bioavailability (13), proteinase inhibitors-containing formulations (14), effective doses (15) and frequent treatment schedules (16). Furthermore, the existence of contradictory outcomes in terms of efficacy when EGF is topically administered (17) has hindered its clinical use.

Endogenous EGF is likely to exert a profound physiological role in a number of epithelial tissues in adult organisms. In addition, the parenteral administration of EGF in pharmacological concentrations has shown prophylactic and/or therapeutic effects in different *in vivo* models. In this review we summarize

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some relevant evidence on the physiological role of the endogenously secreted EGF, and discuss the potential clinical benefits of the parenteral use of EGF.

Is endogenous EGF a physiologically relevant factor?

EGF in embryonic and neonatal life

EGF is considered an embryotrophic factor and is known to enhance mitogenesis, development and implantation in several animal species (18).

The notion that EGF participates in the epigenetic regulation of organ growth and differentiation primarily emerged from detecting EGF mRNA and/or EGF in embryonal or neonatal tissues and in amniotic fluid. EGF has been considered an autocrine factor acting in the regulation of early placental growth (19), in the formation of mesenchymal cell condensates and in the morphogenesis of many organs, including teeth, brain, the male reproductive tract, skin, the gastrointestinal tract and in cardiac differentiation (20-25). Furthermore, EGF levels in amniotic fluid seem to reflect maturational events as it has been demonstrated that EGF levels increase rapidly in late human pregnancy and that intrauterine growth restriction is associated with lower EGF levels in the amniotic fluid (26).

The induction of EGF autoantibodies has provided direct evidence in this scenario. The offspring pups of EGF-autoimmune rats showed a significant increase in neonatal mortality (27). A similar effect was observed in mice upon pregestational sialoadenectomy (28). In both animal species, a lower birth weight was observed in the surviving pups. These animals had immature lungs with decreased net weight accompanied by a reduction in the surfactant production with concomitant respiratory distress syndrome. Other data confirm that EGF is a powerful agent in stimulating the biochemical development of the fetal lung (29).

The newborn pups from the EGF-immunized rats also showed dry and wrinkled skin. This appearance was supported by finding a thinner skin with poorly developed hair follicles. Liver was also affected in these EGF-deficient animals so that the relative organ weight was significantly lower in the EGF-deficient pups. In addition, these livers had a lower portion of cells in the S phase (27).

Other evidences indicate that the EGF present in the milk and colostrum of various species (30-32) is a trophic agent for the gastrointestinal tract and that the growth factor stimulates cellular proliferation, organ growth and functional maturity. The human colostrum contains two kinds of growth factors and one of them is an epidermal growth factor like (EGF-like) growth factor which seems to be involved in gastrointestinal maturation and therefore in promoting baby growth and development (33, 34). Similarly, the milk-borne EGF seems to play an important role in regulating postnatal development in the suckling pig (35).

These data demonstrate that EGF is present in embryonic, fetal and neonatal life and that its deficiency may seriously affect the developmental clock, natural organ morphogenesis and maturation.

EGF in adult life

EGF is locally secreted in an abundant number of tissues in adult animals (36). Recent data support its physiological significance in epithelial cytoprotection, tissue trophism, cell population renewal, and in assisting the healing of epithelial defects (37, 38). In this opportunity we will focus on those organs or systems in which there is mounting evidence suggesting the significance of the parenteral administration of pharmacological doses of EGF in treating experimental or clinical disorders.

I.- Gastrointestinal tissue

The physiological significance of EGF in systems such as the gastrointestinal tract of mature animals is unclear. For this system, a question which remains unanswered is whether luminal EGF has an effect on the intact intestinal mucosa, or whether this EGF exists only to repair mucosal injuries (39). However, in any other system the role of endogenous EGF has been so extensively documented so far as in the gastrointestinal tract.

The gastrointestinal tract is constantly covered with saliva and the EGF of the duodenal Brunner glands (40, 41), and this duodenal EGF is also made continuously available to the liver through portal circulation (42). Although luminal EGF undergoes C-terminal truncation in the gastrointestinal lumen (43, 44), it has been postulated that a significant part of salivary EGF may remain stable and that it has a functional significance (45).

It has been shown that luminal EGF exerts a trophic effect throughout the entire intact gastrointestinal tract of mature animals (46-48), as the salivary hypersecretion elicits a trophic response in the small intestine, whereas stimulants of salivary secretion do not affect intestinal growth when administered to sialoadenectomized animals (49).

The practice of sialoadenectomy in rodents has been a useful tool in studying the role of salivary EGF. In rats, removal of submaxillary glands greatly reduced the rate of ^{3}H-thymidine incorporation into gastric mucosa (50) and delayed the healing of gastric ulcers (51). These evidences received additional support by demonstrating that sialoadenectomy provoked a reduction in the rate of ulcer healing and in the total content of DNA and RNA in the gastroduodenal mucosa due to a significant reduction in the amount of immunoreactive EGF present in the gastric lumen. The mucosal growth parameters were restored by the repeated administration of EGF either orally or subcutaneously (52).

The lack of salivary glands and thus of the major source of EGF, proved to be a condition associated to gastric mucosal damage exacerbation, as judged by the extent of the mucosal damage induced by a variety of agents in different animal models (53). Further evidence indicated that the gastric overexpression of EGF and its receptor are a component of the adaptive cytoprotection mechanism against gastric irritants and necrotizing agents (54).

In line with the above evidences on the role of endogenous EGF in the gastrointestinal tract, salivary EGF deficiency has been reported by Ohmura *et al.* (55) in patients with active peptic ulcer disease.

Furthermore, a marked reduction in the concentration of EGF was found in the gastric juice of both duodenal and gastric ulcer patients in the active stage. This difference disappeared in gastric ulcers after healing was achieved with H_2-antagonist treatment (56). Similar results by this group confirmed this evidence in a larger population.

A decrease in the EGF salivary production has been suggested as a possible mechanism for the increased susceptibility to gastric ulceration in patients with rheumatoid disease plus the sicca syndrome (57). Consistent with this hypothesis Biagini *et al.* (58) have reported an increased occurrence of peptic ulcer in patients with primary biliary cirrhosis and Sjörgen's syndrome, thus highlighting the possible pathophysiological relevance of the underlying exocrine gland defect.

In humans on total parenteral nutrition, or in those with salivary gland atrophy after radiation treatment, the gastrointestinal mucosa atrophies. In both conditions a substantial reduction in the amount of gastric luminal EGF is achieved (59, 60). In addition, the negligible EGF production by the submandibular glands with radiotherapy induced sialoadenitis, is associated to mucositis. These results support the hypothesis that the continual presence of EGF is required to maintain the integrity of the oral and gastrointestinal mucosa in adult organisms.

A significant contribution to unequivocally confirm the role of the endogenous EGF in ulcer healing was provided by Wright *et al.* (61). The authors described the presence of an EGF novel-secreting gland, originated from a stem cell at the bottom of the crypt with an emerging duct that releases mature immunoreactive EGF near the ulcer margin.

II. - Other tissues where EGF seems to be relevant

Hepatic tissue. Although the role of salivary or duodenal EGF in liver physiology is unclear, the data presented below suggest that the endogenously secreted polypeptide acts as a "priming" signal to hepatocyte mitosis after liver injury (62).

The expression of mature EGF in intact or regenerating livers is controversial. However, this growth factor is a potent mitogen for primary cultured hepatocytes and its administration together with insulin or glucagon significantly stimulates DNA synthesis in intact or partially hepatectomized rats (63).

In rats, sialoadenectomy, which causes a major reduction in plasma EGF, also decreases the regenerative response of the liver after partial hepatectomy. In contrast, plasma EGF concentration rises close to 30 % after inducing this procedure. Accordingly, the decrease of the hepatic mass provoked by a one-third hepatectomy increases the EGF concentration per unit liver weight by a factor of 3. On the other hand, it is known that norepinephrine secretion dramatically increases after partial hepatectomy, thus stimulating EGF secretion by the Brunner glands, which may further increase the amount of EGF entering the liver after partial hepatectomy. In this scenario, rapid tyrosine phosphorylation and down-regulation of the EGF receptor occur shortly later, suggesting that EGF may play a mitogenic role early in the regenerative process of the liver (42, 64).

Finally, it should be pointed out that there is recent evidence suggesting that EGF may prevent lipid peroxidation, thus acting as a hepatoprotective agent *in vitro* (65) and *in vivo* (66). On the basis of these observations it is conceivable that the continual EGF inflow from the duodenum to the liver is likely an important element in ordinary liver detoxification functioning.

Renal tissue. The biological significance of renal EGF is uncertain. This polypeptide growth factor is produced in the kidney and excreted in the urine. The major source of renal EGF are the tubular cells, where it is considered an important growth factor in (i) the development of the tubular segment, (ii) the onset of renal hypertrophy, (iii) maintaining the histological structure and functions of renal tubuli, (iv) promoting tubular epithelial cell regeneration (67, 68). Recent data confirm that EGF is a critical element involved in renal repair, namely in the regeneration of tubular cells and in the prevention of the irreversible tubular injury (67).

Fundamentals for the parenteral use of EGF. Clinical applications

As insights have been gained while identifying the molecular basis of some diseases, along with an understanding of the molecular pharmacology of polypeptide growth factors, novel therapeutic methods forwarded to prevent tissue injury, or to accelerate tissue repair have appeared, thus lessening morbidity and mortality.

The parenteral administration of exogenous natural or recombinant EGF has proved to be an attractive candidate medication for different diseases or acute organic failures. In the gastrointestinal tract, for example, the pharmacological effects of EGF can be summarized as follows.

In animal models EGF has been shown to prevent (69-72) and heal (73, 74) experimentally induced gastrointestinal ulcerations. Indeed, these effects are clearly seen when the compound is administered systemically (39).

The potent inhibitory action of EGF on gastric acid secretion has been exploited clinically in patients with Zolloinger-Ellison disease (75). This EGF-mediated effect has also been confirmed in patients with duodenal ulcers (76).

A clinical trial in patients with gastric ulceration was conducted using natural human EGF (purified from urine). EGF (approximately 6 ug) was intravenously injected twice a week for 8 weeks. The healing rate of the EGF-treated group was significantly faster than that observed in the group that received the placebo (77).

The first clinical application of EGF in hastening repair of a severely damaged intestinal mucosa was reported in 1991 (78). A 9-month-old girl with extensive necrotizing enteritis received a continuous intravenous infusion of EGF at 100 ng/kg/h for 6 days. Serial small intestinal biopsies revealed a dramatic increase in crypt-cell mitotic activity, which accompanied a rapid recovery of villus architecture.

EGF has been used to treat three children affected by congenital microvillus atrophy. The growth factor was intravenously infused at a dose of 100 ng/kg/h for two 6-day periods with a 5-day rest period. The response was assessed after the EGF therapy by examining the small bowel mucosal morphology, epithelial cell kinetics as well as functional absorptive parameters. The EGF treatment provoked an increased crypt-cell production rate, accompanied by an increase in both the crypt cell population size and rate of the proliferating fraction (79, 80).

Other possible gastrointestinal applications

According to preclinical data, EGF might find a clinical application in the treatment of colonic lesions such as those associated with inflammatory bowel disease (81).

The existence of a subset of EGF-deficient patients is supported by recent observations conducted in patients with active or healing ulcers (55). Furthermore, luminal gastric EGF deficiency has been reported as a consequence of salivary gland atrophy or total parenteral nutrition. In these conditions the mucosa is atrophied or severely damaged, thus the parenteral administration of EGF might exert a beneficial trophic effect by restoring the morphological and functional integrity of the mucosa. Romano *et al.* (82) reported lower duodenal concentration of endogenous EGF in cirrhotic patients which may partially explain the increased susceptibility of these patients to duodenal ulcer. In this model, the use of a rational EGF administration program may prevent or decrease mucosal damage.

An additional suggestion is to exploit the EGF trophic properties in novel nutritional strategies for parenteral or enteral nutrition (83).

Studies in animal models suggest the feasibility of using EGF after massive small bowel resection. The subcutaneous administration of EGF after bowel resection, resulted in a significant increase of animal weight, small bowel weight and length, and mucosal thickness (84). These data suggested that EGF augmented the mucosal hyperplastic response after bowel surgery, whereas at the same time it could be of nutritional benefit to the host through its enhancement of the normal adaptive response to intestinal resection.

Another potential use for the parenteral administration of EGF has emerged from preclinical data, indicating that the subcutaneous administration of EGF might reduce sclerotherapy-induced oesophageal damage by significantly stimulating mucosal proliferation (85). On the other hand, different pancreatic cell types were found in a hyperproliferative state after the EGF subcutaneous infusion (10 μg/kg/h for 7 days) to mice (86). However, further experiments are needed to confirm the possibility of using EGF to stimulate pancreatic repair after traumas.

The role of the parenterally administered EGF in other tissues

Liver

Certain data suggest that EGF or EGF-like factors may be mechanically involved in the early "promotion" stage during the pre-replicative phase of liver regeneration (62). Furthermore, the administration of EGF together with insulin or glucagon increases liver DNA synthesis in normal or hepatectomized rats. These data indicate that EGF may not be a major participant of liver regeneration *in vivo*, but that it has a strong effect on hepatocyte replication when injected to the whole animal whether intact or hepatectomized (63). For the case of newborn rabbits, the parenteral administration of EGF provoked a significant increase in liver weight (87).

We have recently demonstrated that the prophylactic, intraperitoneal administration of EGF to CCl_4-intoxicated rats significantly prevented lipid peroxidation and liver inflammation according to the extent and intensity of the parenchymal necrosis, all of which is associated to this hepatitis-like model. This EGF novel effect was observed in a dose dependent fashion (66).

Lungs

EGF (88) and its receptor (89) are present in the lung before birth and the parenteral administration of EGF in pharmacological doses, has shown to induce func-

tional and morphologic lung maturation in different animal species.

The treatment of fetal rhesus monkeys with recombinant human EGF (66 micrograms/kg simultaneously into both the amniotic fluid and the fetal abdominal cavity for 7 days) demonstrated that EGF can induce maturation of the tracheobronchial secretory apparatus. The epithelium was found to be taller and to contain a greater proportion of secretory cells in the tracheas of EGF-treated monkeys. Besides, more secretory product was found to be stored in the epithelium and submucosal glands. The secretory product was also increased both in the lung lavage and amniotic fluid of the EGF treated monkeys (90).

The exogenous administration of EGF in the last trimester of pregnancy proved to accelerate structural and functional cytodifferentiation of the alveolar type II cells in fetal primates. These maturational changes occur in the absence of significant alterations in overall lung growth or morphogenesis of the gas exchange area (91).

Premature rhesus infants, delivered at 78 % of gestation were treated in utero with EGF, which resulted in consistent histologic and biochemical maturation of their lungs. The surfactant apoprotein A concentration and lecithin to sphingomyelin ratio were both significantly higher in the amniotic fluid of the EGF-treated group, indicating advanced biochemical maturation; although birth weight was not affected by EGF exposure, adrenal and gut weights, standardized for body weight, were significantly increased. Histologic studies showed advanced cellular maturation with increased parenchymal airspace in the EGF-treated group as compared with the control group (92). It should be pointed out that previous observations from the last decade had already demonstrated that administering pharmacological doses of EGF (93, 94) stimulates functional and morphologic lung maturation, in rabbit and sheep fetuses.

These data unequivocally demonstrate that the prenatal exposure to EGF promotes the maturation of the lung and markedly attenuates the clinical severity of respiratory disease, at least in animal models of the respiratory distress syndrome (92).

Kidney

Repair and recovery of ischaemic or nephrotoxic acute renal failure (ARF) are dependent upon renal tubule cell regeneration, and EGF is recognized as a potent growth promoter to renal tubule cells (67). On the other hand, renal immunoreactive EGF levels increase in ischaemic renal failure (95), suggesting its implication in the mechanism of renal repair.

Humes *et al.* (96) were the first to demonstrate that EGF accelerates the repair process of a visceral organ after injury. They evaluated the effect of the administration of exogenous EGF in the regenerative process of the kidney as the recovery of the renal function in the ischaemic injury model in rats. The treatment with EGF produced increases in renal thymidine incorporation compared with non-treated animals at 24, 48, and 72 h after ischaemic injury. This accelerated DNA replicative process was associated with significantly lower blood urea nitrogen (BUN) and serum creatinine levels, and was also associated with a return to near normal BUN and serum creatinine levels in EGF-treated animals, approximately 4 days earlier than in the control animals.

The effect of the exogenous, subcutaneously administered EGF at 20 μg, 2 or 4 h after $HgCl_2$ injection, was studied by Coimbra *et al.* (97) during the recovery phase of the chemically-induced ARF. Exogenous EGF resulted in greater levels of renal ^{3}H-thymidine incorporation into renal proximal tubule cells, as compared with those observed in non-treated animals. This EGF-related acceleration in DNA synthesis was associated with significantly lower peak BUN and serum creatinine levels.

These experiments illustrate that exogenous EGF accelerates the repair process of the kidney and enhances its functional recovery after severe toxic or ischaemic insults. These data also suggest the possibility of using EGF as a promising medication for acute renal failure in humans, which is a life-threatening condition.

Miscellaneous activities

The EGF receptor was identified in Schwann cells as well as in other related structures of the human peripheral nerves (98). Based on the hypothesis that EGF or other EGF-like peptides bind the receptor in peripheral nerves, and that EGF may act as a Schwann cells mitogen *in vivo*, we studied the effect of the EGF intraperitoneal administration in the morphologic and functional restoration of a fully transected sciatic nerve in rats. As judged by the ultraestructural study, it was concluded that EGF prevented Schwann cell degeneration as well as myelin sheeths disorganization. In general, a profound preservation of mitochondria, endoplasmic reticulum and tubular axoplasmatic structures was conferred by the EGF treatment as compared to the control group. Furthermore, EGF completely prevented or significantly delayed the onset of skin ulcers in the affected limb (submitted).

As suggested by animal model experiments, EGF could also be clinically useful in the treatment of certain cancer chemotherapy complications such as oral ulcerative mucositis, and other epithelial toxic reactions related to anticancer drug therapy (99).

The EGF mitogenic activity is generally seen as a major limitation to the clinical use of the growth factor. However, administration of EGF to cancer patients can actually be beneficial. It has been shown that EGF can enhance the sensitivity of cancer cells

and tumor xenografts to antineoplastic agents (100, 101). Thus, EGF sensitizes cancer cells to the effect of chemotherapeutic agents and enhances the clinical efficacy of chemotherapy. On the other hand, it has been observed that EGF does not cooperate with certain well-known mutagenic agents as determined by different *in vivo* mutagenicity tests (José L. Bello, National Institute of Oncology, Havana, personal communication).

Taking into account that (i) systemically administered EGF provokes hypertrophy of the sebaceous glands, follicular sheets, and sweat gland ducts (102), (ii) that remnant epithelial structures and fibroblastoid cells respond to EGF and take part in the healing process of the skin (103), (iii) and that EGF may favour anabolism (104); it is conceivable to consider about the potential benefits of the parenteral administration of EGF as an adjunctive therapy to enhance skin wound healing and to improve the general condition of the patient. Indeed further studies are demanded in this area.

Concerns about EGF treatment

The fact that there is no EGF specificity of activity for any tissue so that an EGF mediated pharmacological response may be achieved in many cells expressing the EGF-receptor, has called for caution in relation to potential side effects. However, recent experimental data indicate that the effects of the parenteral long term administration of high doses of EGF are self-limited, non-progressing, treatment and dose dependent, and reversible after suspending the administrations (José L. Bello and J. Berlanga unpublished data, and 105-108).

The major concern in the clinical use of EGF has been historically associated to its promoter effect in chemically and biologically-initiated experimental tumors. On the other hand, clinical evidences indicate that the EGF receptor is overexpressed in many, if not all, human epithelial derived tumors, whereas this overexpression is considered a poor prognostic marker (109). However, no data suggest that EGF overexposure *in vivo* is sufficient to initiate malignant transformation; on the contrary, as stated above, EGF has been shown to sensitize cancer cells to chemotherapy, to have a potent cytoprotective effect *in vivo*, and to prevent chemically induced mutations in mice. The role of EGF in experimental carcinogenesis was recently reviewed by our group (110) and its reading is encouraged, since this topic is not the main goal of this article.

The careful selection of patients on the basis of the risk-benefit ratio should always be considered prior to EGF administration, whereas EGF treatment is expected to be indicated for acute pathologies requiring short term administrations or for life-threatening diseases.

In summary, clinical and preclinical data highlight the potential benefits of the parenteral administration of EGF for certain pathological conditions in neonates and adults. The long-term toxicological assessment conducted so far suggests that EGF does not initiate cellular malignant transformation. Moreover, EGF systemic administration should be preceded by an exhaustive clinical monitoring.

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