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FUNCTIONAL IMPAIRMENT IN SUBMANDIBULAR GLAND OF RATS INDUCED BY 5-FLUOROURACIL AND CALCIUM LEUCOVORIN

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ABSTRACT

One of the main clinical problems during chemotherapy is the occurrence of severe systemic toxicities, including those related to the stomatognathic system, which contribute to reducing the patient's quality of life. The most frequent oral complications are mucositis, dysgeusia, inflammation, gingival bleeding and decreased salivary flow or hyposalivation, a factor that predisposes to xerostomia, and other local complications that alter the homeostasis of the system.The purpose of this study was to evaluate the functional activity of salivary glands in Wistar rats subject to chemotherapy by measuring salivary flow, glycogen levels and glandular tissue response to autonomic nervous system agonists.

Five experimental groups were used: 1) Control group fed "ad libitum"; 2) 5-fluorouracil (20mg/ kg body weight); 3) Calcium leucovorin (10 mg/kg body weight); 4) 5-fluorouracil + calcium leucovorin (20 and 10 mg / kg, respectively) by intraperitoneal injection for five consecutive days and 5) control with paired diet. Groups 1 and 5 did not receive drugs. Treatment with fluorouracil + leucovorin produced an increase in stimulated salivary flow and a higher response to increasing doses of beta agonists compared to other experimental groups. In both groups treated with cytostatic drugs, blocking of glycogen consumption at the end of the experimental period was observed. Our work suggests that salivary secretion may be affected by a dual mechanism: the first would be toxicity induced by 5-FU, which would cause depression of the process of glucose utilization. The second mechanism would affect the sympathetic autonomic reflex arc. In this instance, the synergistic action of 5-FU + LV would have a negative effect on the nerve activity with a reduction of salivary secretion. This would explain the hyposalivation, cited by several authors in patients undergoing the 5-FU+LV scheme in the treatment of colon carcinoma.

Key words*: Autonomic dysfunction - cytostatics drugs - salivary glands - glycogen.*

ALTERACIÓN FUNCIONAL EN GLÁNDULA SUBMANDIBULAR DE RATAS INDUCIDA POR 5-FLUOROURACILO Y LEUCOVORINA CÁLCICA

RESUMEN

Uno de los principales problemas clínicos durante la quimioterapia es la aparición de graves efectos tóxicos sistémicos, incluidos los relacionados con el sistema estomatognático, que contribuyen a la reducción de la calidad de vida del paciente. Las complicaciones orales más frecuentes son la mucositis, disgeusia, inflamación, sangrado gingival y la disminución del flujo salival o hiposalivación, un factor que predispone a la xerostomía, y otras complicaciones locales que alteran la homeostasis del sistema. El objetivo de este estudio fue evaluar la actividad funcional de las glándulas salivales de ratas Wistar sometidas a quimioterapia, a través de la medición del flujo salival, los niveles de glucógeno y la respuesta del tejido glandular a agonistas del sistema nervioso autónomo.

Se utilizaron cinco grupos experimentales: 1) Control con alimentación "ad libitum"; 2) 5 - fluorouracilo (20 mg / kg de peso corporal); 3) Leucovorina cálcica (10 mg/ kg de peso corporal); 4) 5 - fluorouracilo + leucovorina cálcica (20 y 10 mg / kg, respectivamente) por vía intraperitoneal durante cinco días consecutivos, y 5) control con dieta apareada. Grupos 1 y 5 no recibieron drogas. El tratamiento con 5 - fluorouracilo + leucovorina produjo un aumento de flujo salival estimulado y una mayor respuesta a dosis crecientes de agonistas beta en comparación con otros grupos experimentales. En ambos grupos tratados con citostáticos, se observó bloqueo del consumo de glucógeno al final del período.

Nuestro trabajo sugiere que la secreción salival puede estar afectada por un doble mecanismo: el primero sería la toxicidad inducida por 5-FU que causaría depresión del proceso de utilización de la glucosa. El segundo mecanismo afectaría el arco reflejo autónomo simpático. En este caso, la acción sinérgica de ambos fármacos de 5-FU + LV repercutiría negativamente sobre la actividad nerviosa con una reducción de la secreción salival. Esto explicaría la hiposalivación citada por varios autores en pacientes sometidos al esquema 5-FU + LV en el tratamiento del carcinoma colorrectal.

Palabras clave: *Disfunción autonómica - drogas citostáticas, glándulas salivales - glucógeno.*

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INTRODUCTION

Salivary gland function plays an important role in oral health[1].

The major salivary glands are paired submandibular, sublingual and parotid glands that work in concert with hundreds of minor salivary glands located throughout the region. Each gland has a unique combination of mucous or serous acinar cells, which are responsible for synthesizing protein components of saliva and transporting water and electrolytes. Branching ducts within major salivary glands finalize the electrolyte composition of saliva and deliver it to the mouth[2].

The submandibular glands contribute approximately two-thirds of the unstimulated saliva volume, whereas the parotid glands contribute most of the stimulated saliva volume[3].

The autonomic nervous system predominantly regulates salivary gland secretion[4].

During the chemotherapy period, the occurrence of severe systemic toxicities is one of the major clinical problems, among which are those related to the stomatognathic system, which contribute to reducing the patient's quality of life[5,6].

The most frequent oral complications are mucositis, dysgeusia, inflammation, gingival bleeding and decreased salivary flow or hyposalivation, a factor that predisposes to xerostomia, and other local complications that alter the homeostasis of the system[7].

5-fluorouracil (5-FU) is a drug that is active against a broad spectrum of solid tumors and is the only agent used preferentially in standard therapies in metastatic colorectal cancer[8]. The administration of 5-FU and leucovorin (LV) for the chemotherapy treatment of colorectal carcinoma has shown a significant increase in the rate of survival[9]. 5-FU is a synthetic analogue of uracil. Its mechanism of action is inhibition of the thymidylate synthetase enzyme which causes disruption of DNA synthesis and subsequent cell death[10].

Calcium leucovorin is used in combination with 5-fluorouracil to increase its antineoplastic effect, since Calcium leucovorin allows it to remain longer within the cancerous cell[11].

The rat submandibular gland is a useful model for studying the physiopathology of salivary secretion *in vivo* because of its rich adrenergic innervations and its capacity to use carbohydrates as a source of energy[12-15]. In this context, the purpose of this study was to evaluate the functional activity of submandibular glands (SMG) in Wistar rats subject to chemotherapy, by measuring salivary flow, glandular response to autonomic nervous system agonist drugs and tissue glycogen levels.

MATERIALS AND METHODS

Male Wistar rats (250±50 g), 100±120 days old, located in individual metabolic cages under 12-hour light-dark periods were used. The animals were treated as recommended by the NIH GUIDE and the protocol was approved by the Bioethics Committee of the UNC Medical School.

The drugs used were 5 fluorouracil (TRIOSULES 500 mg solution for injection / Microsules Argentina Laboratory) and Leucovorin (LEUCOCALCIN 50 mg, lyophilized injection/ Kampel Martian laboratory).

The animals were divided into five experimental groups:

1) Control group (C): control rats without cytostatic treatment, fed "*ad libitum*".
2) 5-FU group: each animal was injected with a daily i.p. dose of 20 mg of 5-FU/kg body weight for five consecutive days.
3) LV group: each animal was injected with a daily i.p. dose of 10 mg of LV/kg body weight for five consecutive days.
4) 5-FU+LV group: each animal was injected with a daily i.p. dose of 10 mg of LV/kg body weight, thirty minutes prior to another i.p. injection of 20 mg of 5-FU /kg body weight for five consecutive days.
5) Paired diet group (PD): untreated animals which were given the average daily intake of groups 5-FU, LV and 5-FU+LV for five consecutive days (paired diet).

This group was intended to exclude the effect of reduced intake by action of drug treatment on the functional activity of the SMG.

The animals in the five groups were maintained in a temperature controlled room (24 ± 2.0 ºC) and provided with food and water until 18 hours before the experiments when food, but not water, was withdrawn in order to carry out the experimental activity under the same baseline conditions. The experiments were performed on the seventh day, after recording each animal's body weight.

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Measurement of body weight and food intake

In all groups, daily body weights and food intake were recorded at 7:00 am. Food intake was measured to the nearest 0.1 g by weighing the food basket and the amount of spillage.

Determination of the stimulated salivary flow

In all groups, the submandibular secretory response was analysed. Rats were anaesthetized through a cannula inserted into the jugular vein after induction with ether. A tracheal tube allowed free pulmonary ventilation. Body temperature was measured with a rectal thermometer and maintained at 37.5 °C. Secretory ducts from both submandibular glands were exposed and cannulated with fine glass tubes that gave about 45 drops/ml of distilled water[16, 17].

The nervous control of saliva secretion in salivary glands is carried out by both branches of the autonomic nervous system working synergically, therefore methacoline was administered to stimulate salivary output, and isoproterenol to induce the discharge of stored proteins. Secretory responses were obtained by injecting (*i.p.*) isoproterenol and methacoline jointly (5 mg/kg body weight each dissolved in isotonic saline). These dosages were comparable to those used by other authors in their studies of saliva secretion in rats and the volumes injected ranged from 0.8 to 1.0 ml, depending on the animal's body weight. Saliva was collected for 20 min (from the moment the first drop appeared at the tip of the cannula) into pre-weighed plastic tubes and kept on ice. The volumes of saliva secreted were determined assuming a specific gravity of 1.0 as µg of saliva per mg of dry submandibular gland tissue (µg saliva/mg of dry tissue). After stimulation, the submandibular glands were dissected, dried at 37 °C for 72 h and weighed[18-20].

Dose-response curves

The adrenergic and cholinergic secretory response was analysed in all groups of rats. After the same procedure for salivary secretion, adrenergic secretory responses were obtained by injecting, through a femoral cannula, increasing doses (2.0, 3.0, 10.0, 30.0 mg/kg body weight) of isoproterenol. The saliva was collected on aluminium foil and weighed. The secretory response to low doses (up to 10 mg/kg) stopped within the 3 min following the injection of each dose (3-min secretory response). The 3-min response was recorded for all doses. For doses greater than 10 mg/kg, secretion continued after 3 min and collection was continued until it had stopped (total secretory response).

In another group of animals, the cholinergic response was analysed by injecting, through a femoral cannula, increasing doses (1.0, 3.0, 10.0, 30.0 mg/kg body weight) of methacoline[21, 22].

Determination of tissue glycogen

Some strips were used for glycogen determination immediately after killing the animals (time 0), while others were analyzed after 60 minutes incubation in a Krebs Ringer Bicarbonate (KRB) glucose-free solution (time 60). Tissue glycogen was analyzed using the Johann and Lentini method[23].

All experimental groups were processed similarly, but with the addition of autonomic nervous system agonists.

Statistical analysis

The results were analyzed by means of the paired Student's t-test, setting the value of $p<0.05$ for statistical significance. To calculate the average value of the secretory response for each dose of isoproterenol and methacholine, the volume of saliva produced by each submandibular gland was taken separately.

RESULTS

The groups treated with cytostatic drugs significantly reduced daily food intake compared to group C, with subsequent loss of body weight. In addition, animals showed other signs of physical deterioration such as diarrhea and limb and nasobuccal bleeding. The group treated with LV did not differ from group C, while the paired diet group showed an expected loss of body weight. Gland weight of PD, 5 FU and 5 FU+ LV, both fresh and dry, decreased significantly compared to C and LV, the effect observed in 5FU+LV being higher (Table 1).

The flow rate stimulated by combined doses of isoproterenol and methacholine was greater in the 5-FU + LV group than in the other four groups ($p<0.01$). (Fig.1).

Secretory activity in response to increasing doses of isoproterenol showed variations in the different experimental groups.

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At doses of 2 µg/kg of body weight, there was no change in secretory responses, while at higher doses, differences were found. The greatest effect was expressed in the groups treated with oncologic drugs compared to C and LV groups. The 5- FU group showed a similar response to that of PD. The 5-FU+ LV group had a significant increase of the secretory response in relation to other groups. (supersensitivity) (Fig. 2)

The metacholine dose-response curves showed no change in the different experimental groups (Fig. 3). Despite the increase in basal glycogen concentration (time 0) observed in the two groups treated with cancer drugs, its concentration remained unchanged at the end of the experimental period (time 60). In contrast, in groups C, LV and PD, glycogen concentration decreased significantly after 60 minutes' incubation in KRB (Fig. 4).

The addition of either beta adrenergic agonist or parasympathetic agonist to the incubation medium did not change the situation observed for groups C, LV and PD, but resulted in marked glycogen consumption in the groups treated with cytostatics (Figs. 5a - 5b).

Table 1: Body weight and gland weight in the different experimental groups.

Experimental group	Body weight (g) weight (mg)	Wet glandular weight (mg)	Dry glandular
Control	356±21	216± 8	46±2
Paired diet	306±4 (*)	163±3 (***)	39±0.6 (***)
LV	364±17	221±6	45±2.3
5 FU	305±12 (*)	166±5 (***)	36±2 (***)
5 FU +LV	291±15 (**)	150±5 (***) (#)	34±1 (***) (#)

(*): p< 0.05. Final body weight of animals with paired diet and treated with 5-FU vs. Control;
(**): p< 0.01: Animals treated with 5-FU + LV in relation to Control;
(***):p<0.001: Fresh and dry gland weight of all the experimental groups in relation to Control; (#): p< 0.05: Fresh and dry gland weight of animals treated with 5-FU + LV vs. paired diet. p<0.001.

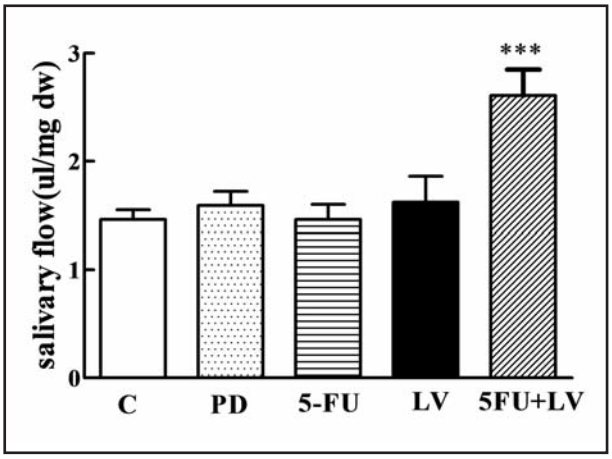

Fig. 1: Salivary flow stimulated i.p. with isoproterenol and methacholine (5mg/kg body weight) in the different experimental groups. (***): p< 0.001: 5-FU+LV vs. C, PD and 5-FU.

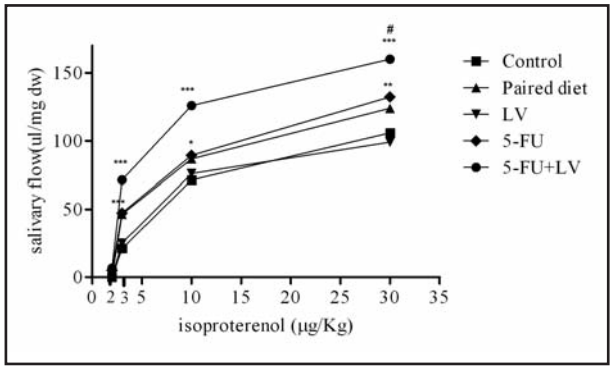

Fig. 2: Dose response curves of salivary secretion in response to increasing doses of Isoproterenol mg/kg body weight in the different experimental groups. (*) p< 0.05: 5-FU and PD + Iso (10 g/Kg body weight) vs. Control; (**) p< 0.01: 5-FU and PD + Iso (30µg /Kg body weight) vs. Control; (***): p< 0.001: 5- FU and PD + Iso (3 µg /Kg body weight) vs. Control; 5-FU + LV + Iso (3-10-30 µg /Kg body weight) vs. Control, PD and 5-FU.

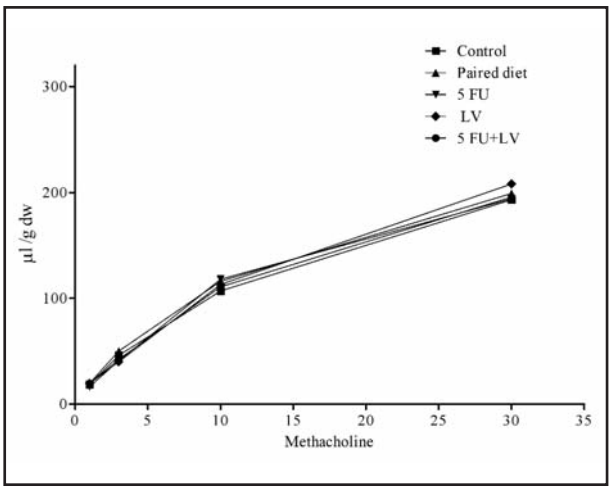

Fig. 3: Dose response curves of salivary secretion in response to increasing doses of methacholine mg/kg body weight in the different experimental groups.

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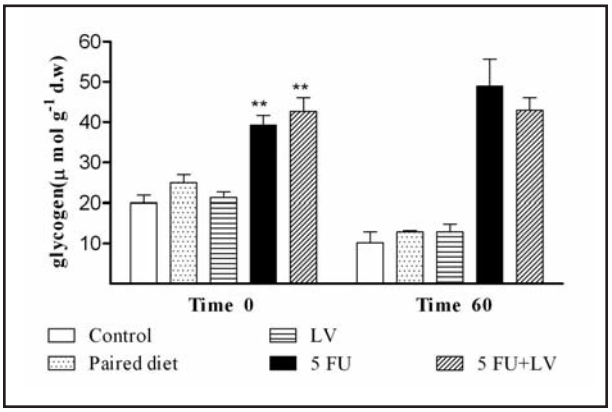

Fig. 4: Glycogen concentration in glands in the different experimental groups. (**): p <0.01: 5-FU and 5-FU + LV (Time 0) vs. Control and PD; Control (Time 60) vs. Control (Time 0). (***) p <0.001 PD (Time 60) vs. PD (Time 0).

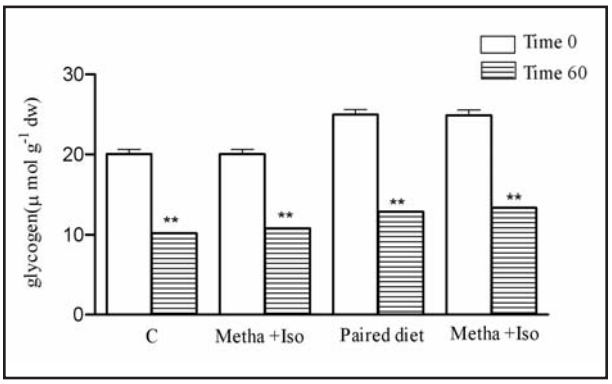

Fig. 5A: Effect of isoproterenol and methacholine on glandular glycogen concentration in groups Control and PD. (**): p< 0.01: Control, Control+ Iso and M and PD, PD + Iso and M (Time 60) vs. (Time 0).

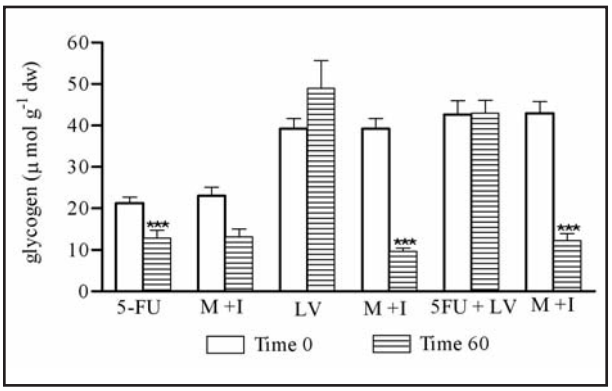

Fig. 5B: Effect of Isoproterenol and methacholine on glandular glycogen concentration in rats treated with cytostatic drugs. (***): p <0.001: 5-FU and 5-FU+LV+ Iso and M (Time 60) vs. (Time 0).

DISCUSSION

This study aimed to evaluate the secretory response *in vivo* of submandibular gland from rats treated with cytostatic drugs through markers such as salivary flow and glycogen consumption[24].

It is known that salivary secretory activity in these rodents can be reproduced by pharmacological stimulation of the autonomic nervous system with agonist drugs[25,26].

In this work, concomitant use of isoproterenol and methacholine caused a significant increase in the production of submandibular saliva in the 5-FU+ LV group compared to other groups.

Since this determination did not show which of the two divisions of the autonomic reflex arc were involved, dose-response curves were made for adrenergic and cholinergic agonists. In response to increasing doses of isoproterenol, the 5-Fu+LV group showed a greater response compared to the other experimental groups. Analysis of the submandibular secretory response in these animals showed β-adrenergic up-regulation, a compensatory phenomenon related to impaired beta adrenergic innervation of the submandibular glands of rats as a result of cytostatic drugs[27,28].

It is probable that these results agree with those obtained by McBride (1987), who demonstrated an increase in the number of beta adrenergic receptors in submandibular gland of rats under the effect of another antimetabolic cytostatic drug such as metotrexate[29].

The results obtained in the presence of 5-Fu were similar to the PD results, and consequently the changes observed in the beta adrenergic response could be attributed to a lower intake rather than to the effect of the drug[30].

It is known that for salivary secretion processes to take place in normal functional conditions, it requires the energy input of metabolic substrates, mainly derived from carbohydrates[31].

Several researchers have established the dependence of the submandibular glands on glycogenolysis and glycolysis mechanisms as the main metabolic sources for salivary secretion to take place[32].

Thus, changes in the glandular glycogen metabolism are used "in vitro" as an indicator of their functional activity[33].

In agreement with these authors, in this work, the groups C, LV and PD showed ability to use glycogen as a metabolic substrate for glandular secretion.

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In contrast, glands isolated from the groups treated with cytostatics showed a higher basal level of glycogen that was not consumed during the experimental period. The addition of isoproterenol and methacoline reversed the initial situation, showing glycogen consumption at the end of the experiment. This might suggest that part of the alterations in the salivary secretion would be due to an alteration of this metabolic substrate by the effect of the cytostatic drugs.

5-fluorouracil could significantly alter the metabolism of carbohydrates. The literature reviewed does not report any use of the same drugs at similar or different doses which would enable comparisons with our results on glycogen consumption.

Our observations allowed us to show depression of gland function by altering the autonomic reflex arc, at significantly lower doses.

5-FU administration would affect glandular activity just blocking the metabolism of carbohydrates while synergic action of both drugs 5-Fu+LV would depress metabolic and nervous activity with hyposalivation.

This would explain hyposialia and associated xerostomy, cited by several authors in patients under 5-Fu+LV scheme for the treatment of colorectal carcinoma.

Ewens reported, among other systemic effects, enlargement of the SMG from the use of these drugs at higher doses. This author also demonstrated a close correlation between drug toxicity, edema, immunosuppression, reduced salivary flow and infection. This is attributed to the immunosuppressive effect of 5-FU, which may induce epithelial damage in the salivary gland and decreased secretion, allowing opportunistic bacteria to settle in its stroma[34].

Although our study does not rule out the presence of bacterial infection from temporary immunological disorders, it suggests that salivary secretion may be affected by a dual mechanism. The first would be toxicity induced by 5-FU, which may cause depression of the process of glucose utilization as the main substrate in the functional activity of the acinar cells. The second mechanism would affect the sympathetic autonomic reflex arc mediated by beta receptors. In this case, the synergistic action of both drugs 5-FU + LV would depress nerve activity with a reduction of salivary secretion. This would explain the hyposalivation, cited by several authors in patients undergoing the 5-FU+LV scheme in the treatment of colorectal carcinoma[35].

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REFERENCES

1. Fox PC, Grisius MM, Bermudez, DK, Sun D. Cytokine mRNA expression in labial salivary glands and cytokine secretion in parotid saliva in Sjogren´s syndrome. Adv Exp Med Biol 1998; 438:909-915.
2. Pinkstaff CA. Serous, seromucous, and special serous cells in salivary glands. Microsc Res Tech 1993; 26:21-31.
3. Ship JA, Fox PC, Baum BJ. How much saliva is enough? "Normal" function defined. J Am Dent Assoc 1991; 122:63-69.
4. Proctor GB, Carpenter GH, Regulation of salivary gland function by autonomic nerves. Auton Neurosci 2007;30, 133:3-18.
5. Petrelli N, Douglass HO Jr, Herrera L, Russell D, Stablein DM, Bruckner HW, Mayer RJ, Schinella R, et al. The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. Gastrointestinal Tumor study Group. J Clin Oncol1 989; 7:1419-1426.
6. Köhne CH, Wils J, Lorenz M, Schöffski P, Voigtmann R, Bokemeyer C, Lutz M, Kleeberg C, et al. Randomized phase III study of high-dose fluorouracil given as a weekly 24-hour infusion with or without leucovorin versus bolus fluorouracil plus leucovorin in advanced colorectal cancer. J Clin Oncol 2003; 21: 3721-3728.
7. Mazzeo M, Linares JA, Campos ML, Busamia B, Dubersarsky C, Lavarda M, Jarchum G, Finkelberg AB. Oral signs of intravenous chemotherapy with 5-fluorouracil and leucovorin calcium in colon cancer treatment. Med Oral Patol Oral Cir Bucal 2009;14: E108-113.
8. Cleary JF, Arzoomanian R, Alberti D, Feierabend C, Storer B, Witt P, Carbone P, Wilding G. A phase I study of 5-fluorouracil, leucovorin and levamisole. Cancer Chemother Pharmacol 1997; 39:300-306.
9. Poon MA, O'Connell MJ, Wieand HS, Krook JE, Gerstner JB, Tschetter LK, Levitt R, Kardinal CG, Mailliard JA. Bio-

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chemical modulation of fluorouracil with leucovorin: confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. J Clin Oncol 1991; 9:1967-1972.
10. Hoff PM, Cassidy J, Schmoll HJ. The evolution of fluoropyrimidine therapy: from intravenous to oral. Oncologist 2001; 6:3-11.
11. Rustum YM, Trave F, Zakrzewski SF, Petrelli N, Herrera L, Mittelman A, Arbuck SG, Creaven PJ. Biochemical and pharmacologic basis for potentiation of 5- fluorouracil action by leucovorin. NCI Monogr 1987; 5:165-170.
12. Ohlin P. Secretory responses of the submaxillary gland in hypophysectomized rats after treatment with thyroxine. Experientia 1968; 24:142-143.
13. Gallara RV, Bellavia SL. Modification of the beta –adrenergic sensitivity of rat submandibular gland by constant light. Arch Oral Biol 1995;40:73-77.
14. Nicolau J, Sassaki KT. Metabolism of carbohydrate in the major salivary glands of rats. Arch Oral Biol 1976; 21: 659-661.
15. Lanfri-Aguilar N, Gallará R, Linares JA, Campos L, Finkelberg A. Restricted diet modifies carbohydrate metabolism in immature rats. Prostaglandins Leukot Essent Fatty Acids 2006; 75: 351-355.
16. Ohlin P. Effects of treatment with isoprenaline or pilocarpine on the secretory responses of the parasympathetically denervated submaxillary gland of the rat. J Oral Ther Pharmacol 1966; 2:376-379.
17. Schneyer CA, Schneyer LH. Electrolyte levels of rat salivary secretions in relation to fluid-flow rate. Am J Physiol 1960;199:55-58.
18. Johnson DA, Cortez JE. Chronic treatment with beta adrenergic agonists and antagonists alters the composition of proteins in rat parotid saliva J Dent Res. 1988; 67:1103-1108.
19. Koller MM, Maeda N, Purushotham KR, Scarpace PJ, Humphreys-Beher. A biochemical analysis of parotid and submandibular salivary gland function with age after simultaneous stimulation with pilocarpine and isoproterenol in females Fischer-344 rats. Arch Oral Biol 1992; 3:219-230.
20. Koller MM, Maeda N, Scarpace PJ, Humphreys-Beher MG. Desipramine changes salivary gland function, oral microbiota, and oral health in rats. Eur J Pharmacol 2000; 408:91-98.
21. Fathi-Moghaddam H, Kesmati M, Kargar HM. The effect of paragigantocellularis lateralis lesion on conditioned place preference (CPP) in presence or absence of α_2 adrenergic agonist (clonidine) in male rats. Acta Physiol Hung 2006; 93:33-40.
22. Martinez JR, Camden J. Volume and composition of pilocarpine-and isoproterenol- stimulated submandibular saliva of early postnatal rats. J Dent Res 1983; 62:543-547.
23. Johann C, Lentini EA . Simultaneous determination of glycogen and lipids from heart muscle. Anal Biochem. 1971; 46:186-187.
24. Ardalan B, Luis R, Jaime M, Franceschi D. Biomodulation of Fluorouracil in colorectal cancer. Cancer Invest.1998; 16:237-251.
25. Gallará RV ,Bellavía SL Modification of the Beta-adrenergic Sensitivity of Rat Submandibular Gland by Constant Light. Arch. Oral Biol 1995;40:73-77.
26. Bellavía SL, Gallará RV. Modification of the b and a2 Adrenergic Sensitivity of Rat Submandibular Gland by Environmental Stimuli and Stress. Arch.Oral Biol 1998; 43: 933-939.
27. Bellavía SL, Sanz EG, Gallará RV, Carpentieri A, Vermouth NT. Effect of sympathetic denervation of the pineal gland on maternal coordination of the circadian rhythm of alpha-amylase in parotid gland from young rats. Arch Oral Biol 1993; 38: 1121-5.
28. Gallara RV, Bellavia SL. Modification of the beta-adrenergic sensitivity of rat submandibular gland by constant light. Arch Oral Biol 1995;40:73-7
29. McBride RK, Harper C, Siegel IA. Methotrexate-induced changes in rat parotid and submandibular gland function. J Dent Res 1987; 66:1445-1448.
30. Elverdin JC, Chiarenza AP, Luchelli MA, Vatta M, Bianciotti LG, Boyer P, Vacas MI. Protein free diet feeding: Effects on sympathetic activity and salivary evoked secretion in the submandibular gland of the rat. Arch Oral Biol 2006; 51:621-628.
31. Nicolau J, Sassaki KT .Metabolism of carbohydrate in the major salivary glands of rats. Arch Oral Biol 1976; 21: 659-661.
32. Nicolau J, de Souza DN, Martins HR. Pilocarpine-induced increases in the activity of 6-phosphofructo-2-kinase and the fructose-2, 6-bisphosphate content of rat salivary glands. Arch Oral Biol 1992; 37:483-487.
33. Nicolau J, Sassaki K.T. Metabolism of carbohydrate in Vitro of the submandibular salivary glands (SMG) from mice injected with isoproterenol. Gen Pharmacol 1983;14: 705-708.
34. Scully C. Drug effects on salivary glands: dry mouth. Oral Dis 2003; 9:165-176.
35. Ewens AD, Mihich E, Ehrke MJ. Fluorouracil plus leucovorin induces submandibular salivary gland enlargement in rats. Toxicol Pathol 2005; 3:507-515.