



Short communication

An effective antidote for paraquat poisonings: The treatment with lysine acetylsalicylate

R.J. Dinis-Oliveira^{a,b,c,*}, H. Pontes^a, M.L. Bastos^a, F. Remião^a, J.A. Duarte^d, F. Carvalho^{a,*}^a REQUIMTE, Department of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal^b Department of Clinical Analysis and Public Health, Center of Research in Health Technologies (CITS)-IPSN-CESPU, CRL, Vila Nova de Famalicão, Portugal^c Institute of Legal Medicine, Faculty of Medicine, University of Porto, Porto, Portugal^d CIAFEL, Faculty of Sport, University of Porto, Porto, Portugal

ARTICLE INFO

Article history:

Received 29 September 2008

Received in revised form 20 October 2008

Accepted 21 October 2008

Available online 6 November 2008

Keywords:

Paraquat

Lysine acetylsalicylate

Treatment

Fibrosis

Survival

ABSTRACT

Sodium salicylate (NaSAL) has been shown to have a multifactorial protection mechanism against paraquat (PQ)-induced toxicity, due to its ability to modulate inflammatory signalling systems, to prevent oxidative stress and to its capacity to chelate PQ. Considering that currently there is no pharmaceutical formulation available for parenteral administration of NaSAL, the aim of the present study was to evaluate the antidotal feasibility of a salicylate prodrug, lysine acetylsalicylate (LAS), accessible for parenteral administrations. PQ was administered to Wistar rats by gavage (125 mg/kg of PQ ion) and the treatment was performed intraperitoneally with different doses (100, 200 and 400 mg/kg of body weight) of LAS. Survival rate was followed during 30 days and living animals at this endpoint were sacrificed for lung, kidney, liver, jejunum and heart histological analysis. It was shown, that the salicylate prodrug, LAS, available in a large number of hospitals, is also effective in the treatment of PQ intoxications. From all tested LAS doses, 200 mg/kg assured animal's full survival. Comparatively to 60% of mortality observed in PQ only exposed animals, the lethality was higher (80%) in the group that received 400 mg/kg of LAS 2 h after PQ administration. The dose of 100 mg/kg of LAS showed only a modest protection (60% of survival). Collagen deposition was observed by histological analysis in survived animals of all experimental groups, being less pronounced in animals receiving 200 mg/kg of LAS, reinforcing the importance of this dose against tissue damage induced by PQ. The results allow us to suggest that LAS should be considered in the hospital treatment of PQ poisonings.

© 2008 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The fatality rate resulting from paraquat (PQ) intoxications is still very high due to the lack of effective treatments (Dinis-Oliveira et al., 2006a, 2008b). In prior studies, we demonstrated, for the first time, that a high-dose therapy with sodium salicylate (NaSAL) has a great potential to constitute a promising treatment of PQ poisonings. It was shown that NaSAL protective effects were associated with the effective inhibition of pro-inflammatory factors such

as nuclear factor kappa-B (NF- κ B), scavenging of reactive oxygen species (ROS), inhibition of myeloperoxidase (MPO), inhibition of platelet aggregation and by preventing death of pulmonary cells through apoptotic pathways (Dinis-Oliveira et al., 2007a,b). More recently, we demonstrated how easily PQ reacts with NaSAL leading to the formation of charge-transfer (CT) complexes with reduced capacity to undergo redox cycling (Dinis-Oliveira et al., 2008a). Considering the relevance of each beneficial effect, it seems reasonable to consider that these findings result from a multi-protective action of NaSAL. Importantly, this treatment was associated with a full survival of the PQ treated rats (extended for more than 30 days) in opposition to 100% of mortality by the day 6 in PQ-only exposed animals. In spite of these encouraging results, there is no pharmaceutical formulation available containing NaSAL for parenteral human administrations. On the other hand, the salicylate (SAL) prodrug, lysine acetylsalicylate (LAS), is readily available as a medicine for intravenous administrations in a large number of hospitals. In our most recent study (Dinis-Oliveira et al., 2008a), we tested a generic formulation of LAS, and we proved that LAS

Abbreviations: ARDS, acute respiratory distress syndrome; ASA, acetylsalicylic acid; COX, cyclooxygenase; CT, charge-transfer; LAS, lysine acetylsalicylate; MPO, myeloperoxidase; NaSAL, sodium salicylate; NF- κ B, nuclear factor kappa-B; PQ, paraquat; ROS, reactive oxygen species; SAL, salicylate.

* Corresponding authors at: REQUIMTE, Department of Toxicology, Faculty of Pharmacy, University of Porto, Aníbal Cunha Street 164, 4099-030 Porto, Portugal. Tel.: +351 222078922; fax: +351 222003977.

E-mail addresses: ricardinis@sapo.pt (R.J. Dinis-Oliveira), felixdc@ff.up.pt (F. Carvalho).

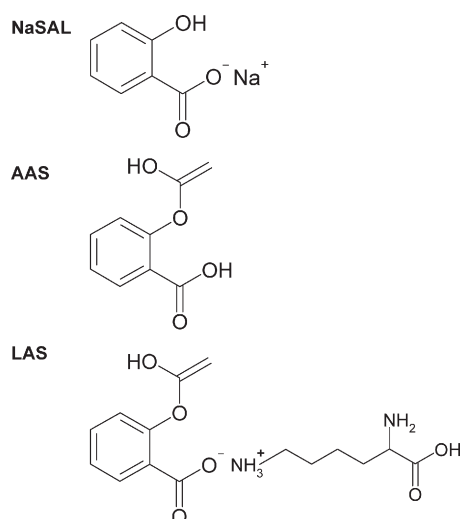


Fig. 1. Chemical structures of sodium salicylate (NaSAL), acetyl salicylic acid (AAS) and lysine acetylsalicylate (LAS).

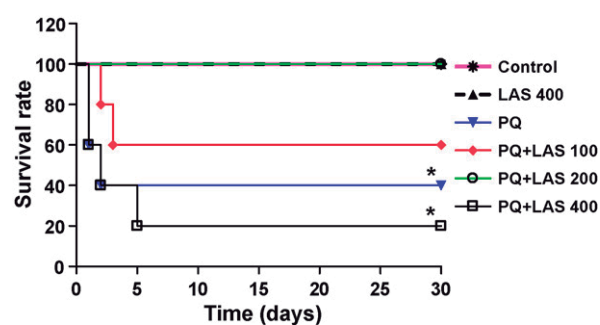


Fig. 2. Percentage of rats surviving in the control, lysine acetylsalicylate (LAS), paraquat (PQ) and paraquat plus lysine acetylsalicylate (PQ + LAS) groups. * $p < 0.05$ versus control, LAS and PQ + LAS 200 groups.

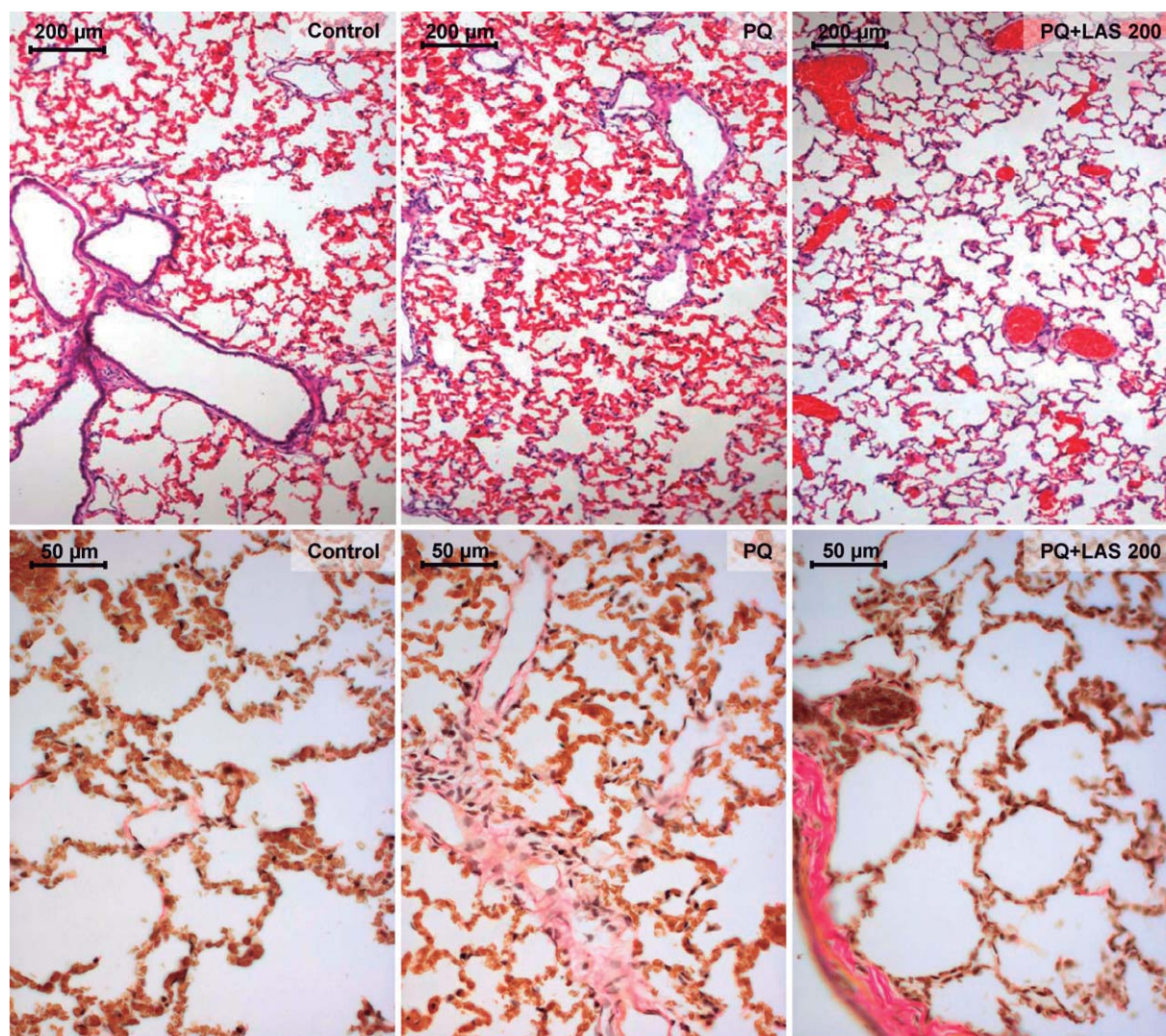


Fig. 3. Light micrographs of lungs from control, PQ, and PQ+LAS 200 groups stained with hematoxylin–eosin (top) and van Gieson (bottom). Lungs from control group exhibited a preserved architecture with low levels of collagen content. PQ-exposed animals showed a regular lung pattern, marked alveolar collapse and the enlargement of alveolar walls, apparently explained by the pronounced vascular congestion and collagen deposition. Animals from PQ + LAS 200 evidenced alveolar walls and spaces comparable to the control group. Vascular congestion was only observed in the large vessels; collagen infiltration was considerably lower in comparison to PQ group.

also reacts with PQ forming CT complexes as NaSAL does. The major difference between NaSAL and LAS is the presence of an acetyl group attached to the phenol ring as in Aspirin® [acetylsalicylic acid, ASA (Fig. 1)]. In contrast to Aspirin®, LAS is a salt compound and therefore is water soluble allowing intramuscular and intravenous injections (Barnéoud and Curet, 1999). In the LAS formulation, 1.8 g of powder prepared by lyophilization is equivalent to 1 g of ASA. ASA, LAS and other acetylsalicylate derivatives can be detected in the plasma only for a short period of time as result of its hydrolysis by carboxylesterases to SAL in plasma, liver, and erythrocytes (Needs and Brooks, 1985; Ulrich et al., 2006; Chyka et al., 2007). Due to this rapid deacetylation, it has been assumed that anti-inflammatory effects of acetylsalicylate derivatives are largely mediated by SAL (Higgs et al., 1987). This assumption receives support by experimental evidence that *in vivo*, SAL and ASA exhibit similar anti-inflammatory potencies (Preston et al., 1989), even though, SAL is not able to acetylate the serine residue of the active cyclooxygenase (COX) site, as occurs with ASA (Vane, 1971; DeWitt et al., 1990). Moreover, in humans the plasma half-life of ASA is about 15 min, whereas for SAL is between 2 and 30 h depending on the ingested dose (Done, 1960; Burke et al., 2006). Considering that NaSAL is the major metabolite of ASA and LAS, and the main responsible for its therapeutic effects, the aim of the present study was to evaluate the feasibility of LAS as an antidote for PQ poisonings. For

this purpose, the higher dose of LAS analysed, was the necessary to reach an equivalent molar concentration of 200 mg/kg of NaSAL, which corresponds to the dose that guaranteed full survival in previous studies (Dinis-Oliveira et al., 2007a,b). It was also a purpose of this study to use oral route for PQ administration, since ingestion is the most common way of intoxication (Dinis-Oliveira et al., 2008b).

2. Materials and methods

2.1. Chemicals and drugs

PQ (1,1'-dimethyl-4,4'-bipyridinium dichloride; molecular mass = 257.2 g/mol), Mayer's haematoxylin solution, eosin Y disodium salt, Weigert's iron haematoxylin solution, van Gieson solution acid fuchsin and di-*n*-butylphthalate-polystyrene-xylene (DPX) mounting medium were all obtained from Sigma (St. Louis, MO, U.S.A.). The saline solution (NaCl 0.9%) and sodium thiopental were obtained from B. Braun (Lisbon, Portugal). Ampoules of LAS (molecular mass = 326.35 g/mol) and water for injections were a generous gift from Labesfal Genéricos (Campo de Besteiros, Portugal). This formulation administered corresponds to the same available for human treatments in Portugal. All the reagents used were of analytical grade or from the highest available grade.

2.2. Animals

A total of 30 rats were included in the study. Male Wistar rats (aged 8 weeks) were obtained from Charles River S.A. (Barcelona, Spain), with a mean weight of 258 ± 23 g. Animals were kept in standard laboratory conditions (12/12 h light/darkness, 22 ± 2 °C room temperature, 50–60% humidity) for at least 1 week

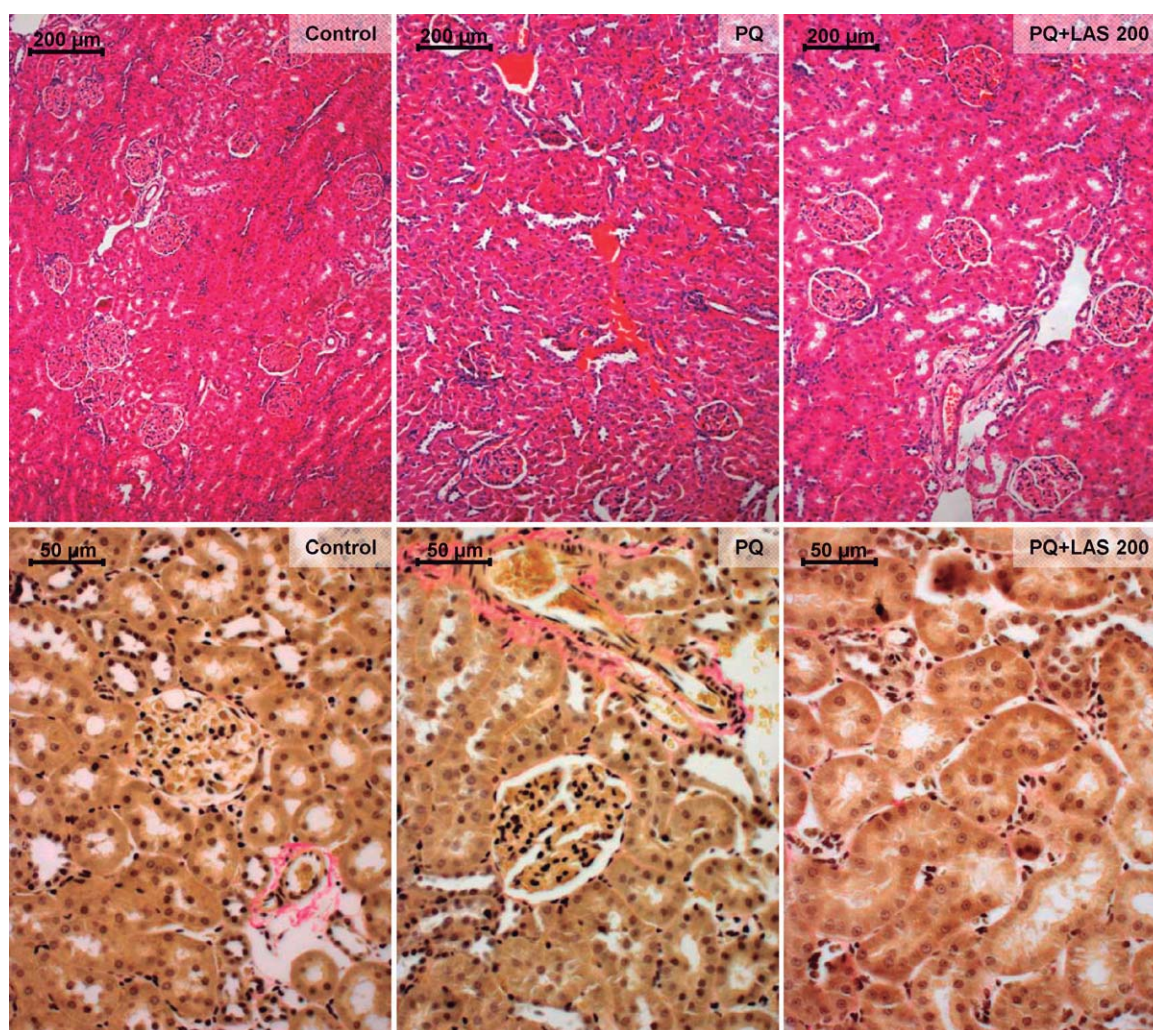


Fig. 4. Light micrographs of kidneys from control, PQ, and PQ + LAS 200 groups stained with hematoxylin–eosin (top) and van Gieson (bottom). All groups evidenced a normal architecture; major alterations in the PQ group were the marked interstitial haemorrhage and the collagen deposition in the interstitial space and surrounding large vessels; collagen deposition in the PQ + LAS group was only noticed in the large vessel walls.

(quarantine) before starting the experiments. Animals were allowed access to tap water and rat chow *ad libitum* during the quarantine period. Animal experiments were licensed by Portuguese General Directorate of Veterinary Medicine (DGV). Housing and experimental treatment of animals were in accordance with the Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory Animal Research (1996). The experiments complied with the current laws of Portugal.

2.3. Experimental protocol for the evaluation of survival rate

For the evaluation of survival rate, 30 animals were randomly divided into six groups of five animals each. Each animal was individually housed during the experimental period in a polypropylene cage with a stainless steel net at the top and wood chips at the screen bottom. Tap water and rat chow were given *ad libitum* during the entire experiment. Treatments in all groups were always conducted between 8:00 and 10:00 a.m. The PQ administered dose (125 mg/kg of PQ ion) corresponds to the oral LD₅₀ reported by several studies (Clark et al., 1966; Kimbrough and Gaines, 1970; Mehani, 1972; Murray and Gibson, 1972; Dinis-Oliveira et al., 2006b). Based on molar concentrations and manufactured instructions, the higher administered experimental dose of LAS chosen was 400 mg/kg, which is equivalent to 200 mg/kg of NaSAL (Dinis-Oliveira et al., 2007a,b). The schedule of LAS administration (2 h after PQ exposure) was chosen taking into account the estimated average arrival time of the patient to hospital, after PQ intoxication. PQ and its vehicle (sterilized water) were administered by gavage in a volume of 0.5 mL/250 g of body weight. The administrations of LAS and its vehicle (0.9% NaCl) were performed intraperitoneally (i.p.) using the same injection volume. The therapeutic regime was the following: (i) control group, *n* = 5: animals were firstly administered with sterilized water and received one administration of 0.9% NaCl 2 h later; (ii) LAS group, *n* = 5: ani-

mals firstly administered with sterilized water and treated with one administration of LAS (400 mg/kg) 2 h later; (iii) PQ group, *n* = 5: animals were firstly intoxicated with PQ (125 mg/kg of PQ ion) and received one injection of 0.9% NaCl 2 h later; (iv) PQ+LAS groups, *n* = 15: animals were firstly intoxicated with PQ (125 mg/kg of PQ ion) and 2 h later, animals were treated with 100 (PQ+LAS 100, *n* = 5), 200 (PQ+LAS 200, *n* = 5) and 400 (PQ+LAS 400, *n* = 5) mg/kg of LAS, corresponding approximately to 50, 100 and 200 mg/kg of NaSAL, respectively. The lethality was registered every day until the day 30 and all surviving animals were sacrificed at this endpoint.

2.4. Surgical procedures and tissue processing for structural analysis

Anesthesia was induced with sodium thiopental (60 mg/kg, i.p.), the trachea was exposed and intubated, and lungs were inflated by administration of the fixative [4% (v/v) buffered formaldehyde; *in situ* fixation]. Lungs, liver, jejunum, kidney and heart were dissected free and submitted to the routine histological procedures for qualitative structural analysis. Briefly, cubic pieces were fixed by diffusion during 24 h and subsequently dehydrated with graded ethanol and included in paraffin blocks. Benzene was used in the transition between dehydration and impregnation. Serial sections (4 µm) of the paraffin blocks were cut by a microtome and mounted on silane-coated slides.

2.5. Staining procedures

The slides were dewaxed in xylene and hydrated through graded alcohols finishing in phosphate-buffered saline (10 mM PBS, pH 7.2). The deparaffinised sections were stained for haematoxylin–eosin and van Gieson protocols. The haematoxylin–eosin staining was performed by immersion in Mayer's haematoxylin

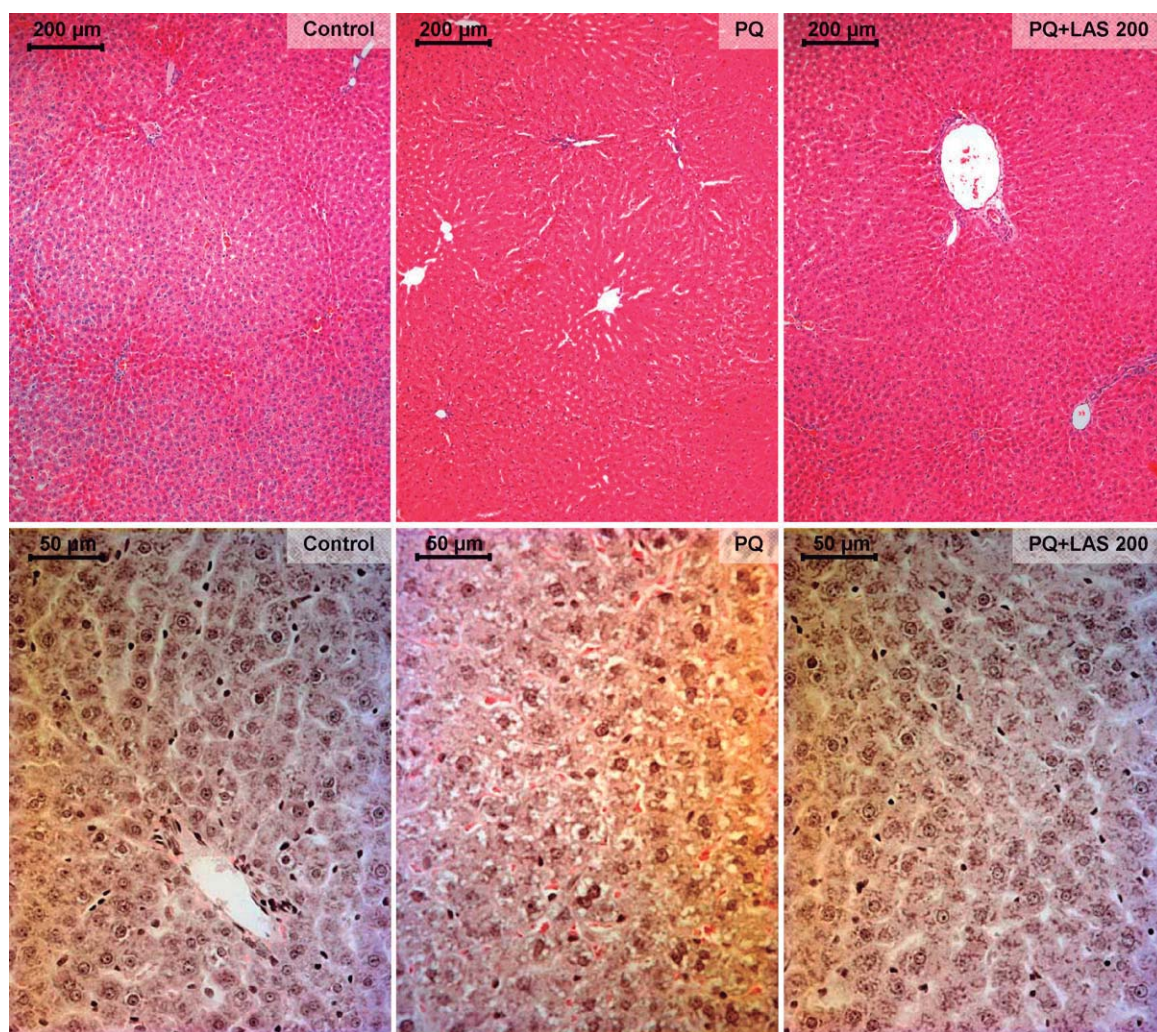


Fig. 5. Light micrographs of liver from control, PQ, and PQ+LAS 200 groups stained with hematoxylin–eosin (top) and van Gieson (bottom). The lobular structure was normal in all groups. In PQ group a marked enlargement of centrilobular sinusoids was observed. An increase of collagen staining was notorious surrounding sinusoids in PQ-exposed animals, which was more pronounced in PQ group.

solution for 3–4 min followed by immersion in 1% eosin solution for 7 min, dehydration with graded alcohols through xylene, and mounting with DPX.

Van Gieson staining (van Gieson, 1889; Weigert, 1904) was applied to evaluate collagen deposition. Briefly, slides were immersed in Weigert's haematoxylin solution for 20 min, washed in tap water for 1 min, differentiated in acid alcohol (1% HCl in 70% alcohol) no more than 5 s, washed again in tap water for 5 min, rinsed in distilled water and immersed in van Gieson's stain for 1 h. Finally, slides were rinsed quickly in distilled water and then in 100% alcohol, cleared and mounted in DPX. Collagen fibers were evidenced by a red staining.

2.6. Statistical analysis

Comparison of the survival curves was performed using the Logrank test. In all cases, *p* values lower than 0.05 were considered as statistically significant.

3. Results

3.1. Survival rate

Rats exposed only to PQ (PQ group) displayed 60% of mortality at the end of 30 days (Fig. 2). Deaths were verified between 24 and 48 h after PQ intoxication. The post-treatment of PQ-exposed rats with LAS (PQ + LAS groups) elicited different results. In the group PQ + LAS 100 it was observed 60% of survival while in the group that received the higher dose of LAS (PQ + LAS 400) only 20% of survival was registered. An increase of the survival rate to 100% was achieved

for a dose of 200 mg of LAS. Therefore, no significant differences were observed concerning the survival rate between the control, LAS and PQ + LAS 200 groups.

3.2. Histological analysis

Lung—major qualitative structural alterations are depicted in Fig. 3. Lungs from control group exhibited a preserved architecture with low levels of collagen content as revealed by van Gieson stain, compatible with a normal pattern. PQ only exposed animals showed a marked alveolar collapse and the enlargement of alveolar walls, apparently explained by the pronounced vascular congestion and collagen deposition. Several macrophage-like cells within alveolar space were also noticed. Animals from PQ + LAS 200 group evidenced alveolar walls and spaces comparable to the control group. Vascular congestion was only observed in the large vessels and collagen infiltration was considerably lower in comparison to PQ group, but higher than the control and LAS groups.

Kidney—major qualitative structural alterations are depicted in Fig. 4. All groups evidenced a normal architecture. Major alterations in the PQ group were the marked interstitial haemorrhage and the collagen deposition in the interstitial space and surrounding large vessels. Collagen deposition in the PQ + LAS 200 group was only noticed in the walls of large vessels.

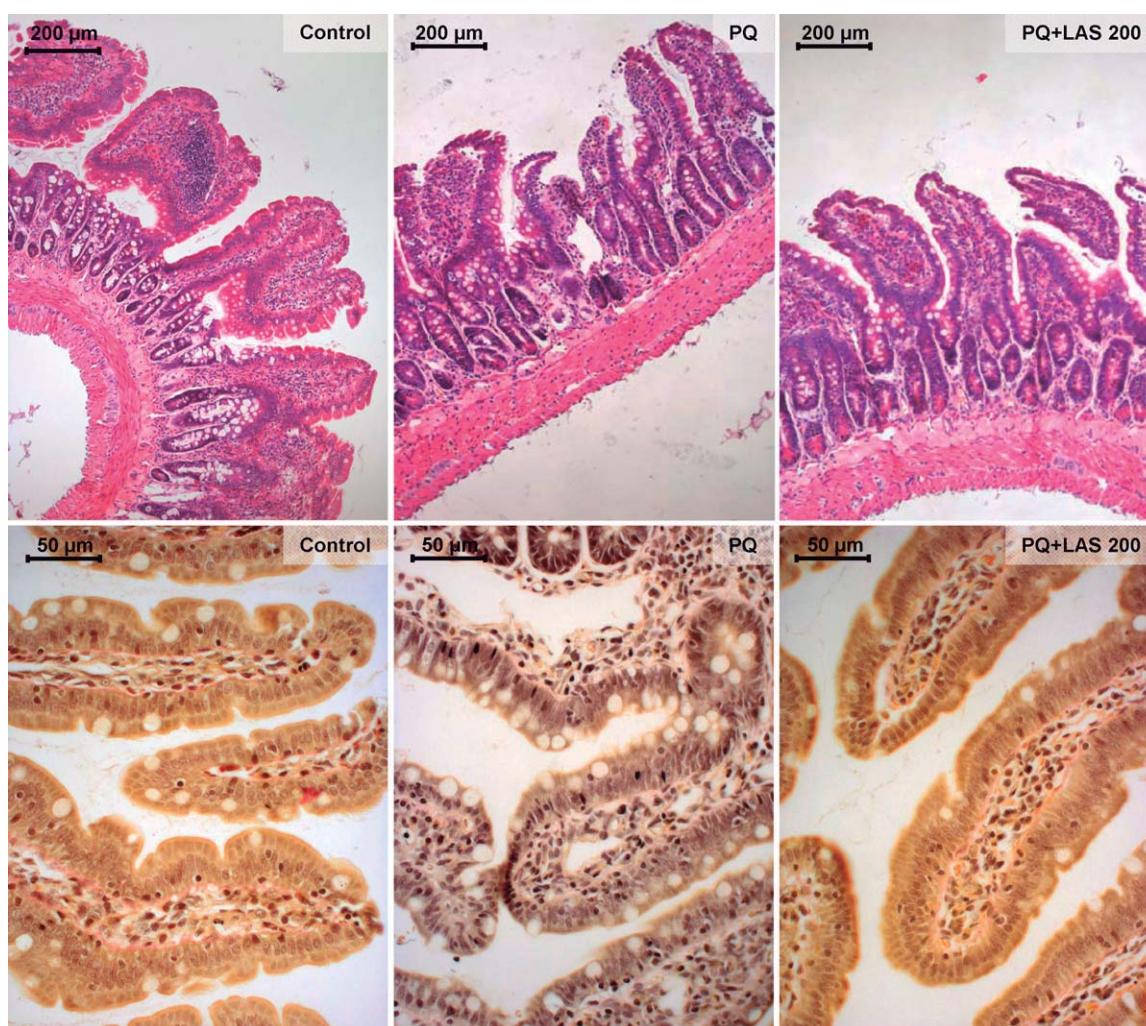


Fig. 6. Light micrographs of jejunum wall from control, PQ, and PQ + LAS 200 groups stained with hematoxylin–eosin (top) and van Gieson (bottom). All groups evidenced a well-preserved architecture without signs of abnormal collagen deposition. In PQ treated groups some confluent translucent areas were detected within villi and neighbouring the crypts.

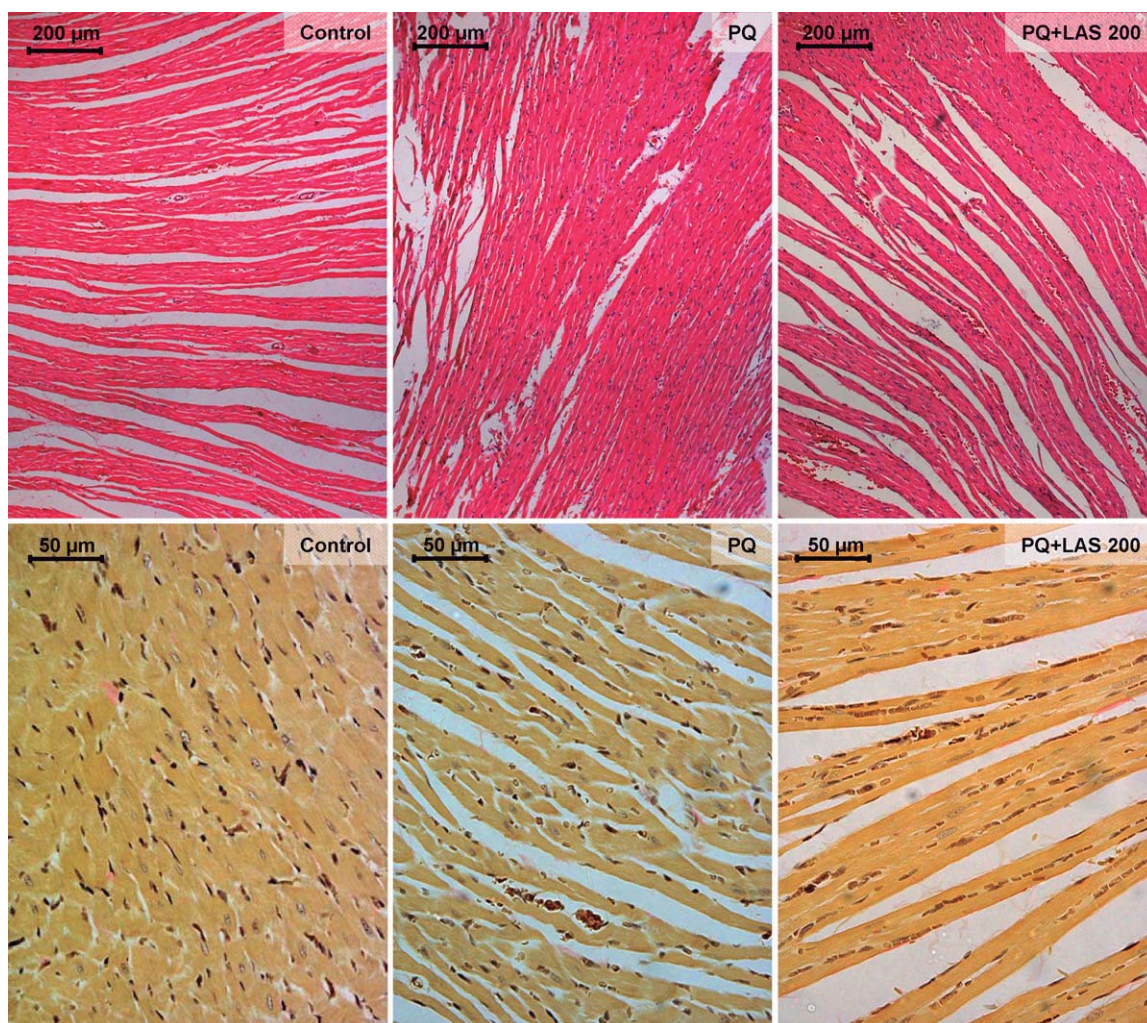


Fig. 7. Light micrographs of heart from control, PQ, and PQ+LAS 200 groups stained with hematoxylin–eosin (top) and van Gieson (bottom). No relevant differences were noticed among groups.

Liver—major qualitative structural alterations are depicted in Fig. 5. The lobular structure was typical in all tested groups. In PQ group, a marked enlargement of centrilobular sinusoids was observed and an increase of collagen staining was notorious surrounding sinusoids in PQ-administered animals, this effect being more pronounced in PQ group.

Jejune wall—major qualitative structural alterations are depicted in Fig. 6. All groups evidenced a well-preserved architecture without signs of abnormal collagen deposition. In PQ treated groups some confluent translucent areas were detected within villi and neighbouring the crypts.

Heart muscle—major qualitative structural alterations are depicted in Fig. 7. No relevant structural changes or differences in the collagen deposition were noticed among all tested groups.

4. Discussion

The results obtained in the present study clearly demonstrate that LAS, in a dose of 200 mg/kg, confers a potent protection against PQ-induced toxicity. In fact, full survival of PQ+LAS 200 group was observed, in opposition to 60% of mortality in PQ-only exposed group. Furthermore, histopathological analysis revealed less structural changes comparatively to PQ group, especially concerning the collagen deposition.

In view of our recent studies, LAS protection could be explained by several mechanisms involving the inhibition of pro-inflammatory factors such as NF- κ B, scavenging of ROS, inhibition of MPO, attenuation of the increase of catalase and glutathione peroxidase activities, inhibition of platelet aggregation, beneficial effects on the apoptotic pathways, and formation of stable CT complexes with PQ (Dinis-Oliveira et al., 2007a,b, 2008a,b).

Noteworthy, LAS was administered 2 h after PQ gavage administration, conferring realism to our study, since medical assistance is only possible a few hours after PQ-poisonings and the majority of intoxications are indeed occurring by ingestion. In addition, with this study we are providing a feasible solution to physicians dealing with the difficult task of treating PQ-intoxicated patients, since the same formulation of SALs, available in the market for hospital intravenous administration was used. However, our results also indicate how difficult it might be to choose the ideal therapeutic dose for clinical practice, since in the PQ+LAS 100 and PQ+LAS 400 groups it was observed 60% and 20% of survival, respectively, which contrasts with the full survival achieved for PQ+LAS 200 group. In fact, if with lower doses of LAS the protective effect might be insufficient, the use of too much higher doses of LAS increases the risk of toxicity. Accordingly to literature human data, the pathophysiological changes attributable to high doses of SALs result in various clinical manifestations depending on plasma concentrations. In adults,

mild toxicity is verified for 300–500 mg/L, moderate toxicity occurs for 500–700 mg/L and severe cases ensue for plasma concentrations higher than 750 mg/L (Done, 1978; Temple, 1981; Proudfoot, 1983). In mild intoxications, side effects such as nausea, vomiting, tinnitus, hyperventilation and respiratory alkalosis can occur. In moderate poisonings, all of the above plus tachypnea, hyperpyrexia, sweating, dehydration, loss of coordination, and restlessness, can take place. In severe poisonings, hallucinations, stupor, convulsions, cerebral oedema, oliguria, renal failure, cardiovascular failure, and coma may be seen together with metabolic acidosis (Proudfoot, 1983; Chapman and Proudfoot, 1989; Yip et al., 1994; Dargan et al., 2002). Taking into account to the above literature data and according to our results, it might be reasonable to assume doses of 200 mg/kg of LAS by intravenous administration as a good starting dose to be tested in human PQ-poisoning treatments. Nevertheless, it should be realized that in our experimental design only a single dose of LAS was given to PQ-exposed animals. In hospital emergency rooms, continuous infusion or repeated administrations are feasible, which may constitute an advantage comparatively to our study, since it may allow to further decrease the doses in acute LAS administrations. It should be noted that roughly 80% to 90% of the SALs in plasma are bound to proteins, especially albumin, at concentrations encountered clinically. Although saturation of the binding sites are likely to occur with such high doses of LAS, a phenomenon that may explains the toxicity (Burke et al., 2006), it is important to note that only the unbound (free) SAL remains in the active state and therefore available to counteract PQ toxic effects.

Structural analysis provided important data to fulfil the aim of this study. Although 40% of survival was achieved in PQ-only exposed group, several and serious structural lesions were observed in organs at the end of 30 days, suggesting that the life quality of these animals is compromised. On the other hand, on rats of the group PQ+LAS 200, not only a full survival was observed but also the structural alterations in the lung, kidney, liver and jejune tissue, were considerably attenuated, especially concerning to collagen deposition. No important structural differences were observed for cardiac tissue in any group, which shows that the heart is the least affected organ as consequence of PQ toxicity.

In conclusion, LAS represents nowadays probably a powerful tool that could be immediately used within hospital premises as an effective antidote for PQ poisonings. The use of pharmacological treatments to prevent PQ toxicity in preclinical trials, if successful, will certainly contribute to lower the morbidity and mortality related to this herbicide, the ultimate goal of our recent studies. The next step is to evaluate this therapeutic approach in humans through clinical trials.

Conflict of interest

None.

Acknowledgements

Ricardo Dinis-Oliveira, acknowledges FCT for his Post-Doc grant (SFRH/BPD/36865/2007) and for the fruitful collaboration of the graduation students Isabel Costa, Diana Félix and Carina Almeida from the Department of Clinical Analysis and Public Health from “Cooperativa de Ensino Superior Politécnico e Universitário

(CESPU)”. This work received financial support of CESPU (project AL/12/2007/CESPU).

References

- Barnéoud, P., Curet, O., 1999. Beneficial effects of lysine acetylsalicylate, a soluble salt of aspirin, on motor performance in a transgenic model of amyotrophic lateral sclerosis. *Exp. Neurol.* 155, 243–251.
- Burke, A., Smyth, E., FitzGerald, G.A., 2006. Analgesic-antipyretic and anti-inflammatory agents: pharmacotherapy of gout. In: Brunton, L.L., Lazo, J.S., Parker, K.L. (Eds.), *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*. McGraw-Hill, New York, pp. 671–716.
- Chapman, B.J., Proudfoot, A.T., 1989. Adult salicylate poisoning: deaths and outcome in patients with high salicylate concentrations. *Q. J. Med.* 72, 699–707.
- Chyka, P.A., Erdman, A.R., Christianson, G., Wax, P.M., Booze, L.L., Manoguerra, A.S., Caravati, E.M., Nelson, L.S., Olson, K.R., Cobaugh, D.J., Scharman, E.J., Woolf, A.D., Troutman, W.G., 2007. Salicylate poisoning: an evidence-based consensus guideline for out-of-hospital management. *Clin. Toxicol. (Phila.)* 45, 95–131.
- Clark, D.G., McElligott, T.F., Hurst, E.W., 1966. The toxicity of paraquat. *Br. J. Ind. Med.* 23, 126–132.
- Dargan, P.I., Wallace, C.I., Jones, A.L., 2002. An evidence based flowchart to guide the management of acute salicylate (aspirin) overdose. *Emerg. Med. J.* 19, 206–209.
- DeWitt, D.L., el-Harith, E.A., Kraemer, S.A., Andrews, M.J., Yao, E.F., Armstrong, R.L., Smith, W.L., 1990. The aspirin and heme-binding sites of ovine and murine prostaglandin endoperoxide synthases. *J. Biol. Chem.* 265, 5192–5198.
- Dinis-Oliveira, R.J., de Pinho, P.G., Ferreira, A.C., Silva, A.M., Afonso, C., Bastos, M.D., Remiao, F., Duarte, J.A., Carvalho, F., 2008a. Reactivity of paraquat with sodium salicylate: formation of stable complexes. *Toxicology* 249, 130–139.
- Dinis-Oliveira, R.J., Duarte, J.A., Sanchez-Navarro, A., Remiao, F., Bastos, M.L., Carvalho, F., 2008b. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. *Crit. Rev. Toxicol.* 38, 13–71.
- Dinis-Oliveira, R.J., Remiao, F., Carmo, H., Duarte, J.A., Navarro, A.S., Bastos, M.L., Carvalho, F., 2006a. Paraquat exposure as an etiological factor of Parkinson's disease. *Neurotoxicology* 27, 1110–1122.
- Dinis-Oliveira, R.J., Sarmiento, A., Reis, P., Amaro, A., Remiao, F., Bastos, M.L., Carvalho, F., 2006b. Acute paraquat poisoning: report of a survival case following intake of a potential lethal dose. *Pediatr. Emerg. Care* 22, 537–540.
- Dinis-Oliveira, R.J., Sousa, C., Remiao, F., Duarte, J.A., Ferreira, R., Sanchez Navarro, A., Bastos, M.L., Carvalho, F., 2007a. Sodium salicylate prevents paraquat-induced apoptosis in the rat lung. *Free Radic. Biol. Med.* 43, 48–61.
- Dinis-Oliveira, R.J., Sousa, C., Remiao, F., Duarte, J.A., Navarro, A.S., Bastos, M.L., Carvalho, F., 2007b. Full survival of paraquat-exposed rats after treatment with sodium salicylate. *Free Radic. Biol. Med.* 42, 1017–1028.
- Done, A.K., 1960. Salicylate intoxication. Significance of measurements of salicylate in blood in cases of acute ingestion. *Pediatrics* 26, 800–807.
- Done, A.K., 1978. Aspirin overdosage: incidence, diagnosis, and management. *Pediatrics* 62 (Suppl.), 890–897.
- Higgs, G.A., Salmon, J.A., Henderson, B., Vane, J.R., 1987. Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and anti-inflammatory activity. *Proc. Natl. Acad. Sci. U.S.A.* 84, 1417–1420.
- Institute for Laboratory Animal Research, Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 1996.
- Kimbrough, R.D., Gaines, T.B., 1970. Toxicity of paraquat to rats and its effect on rat lungs. *Toxicol. Appl. Pharm.* 17, 679–690.
- Mehani, S., 1972. The toxic effect of paraquat in rabbits and rats. *Ain Shams Med. J.* 23, 599–601.
- Murray, R.E., Gibson, J.E., 1972. A comparative study of paraquat intoxication in rats, guinea pigs and monkeys. *Exp. Mol. Pathol.* 17, 317–325.
- Needs, C.J., Brooks, P.M., 1985. Clinical pharmacokinetics of the salicylates. *Clin. Pharmacokinet.* 10, 164–177.
- Preston, S.J., Arnold, M.H., Beller, E.M., Brooks, P.M., Buchanan, W.W., 1989. Comparative analgesic and anti-inflammatory properties of sodium salicylate and acetylsalicylic acid (aspirin) in rheumatoid arthritis. *Br. J. Clin. Pharmacol.* 27, 607–611.
- Proudfoot, A.T., 1983. Toxicity of salicylates. *Am. J. Med.* 75, 99–103.
- Temple, A.R., 1981. Acute and chronic effects of aspirin toxicity and their treatment. *Arch. Int. Med.* 141, 364–369.
- Ulrich, C.M., Bigler, J., Potter, J.D., 2006. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. *Nat. Rev. Cancer* 6, 130–140.
- van Gieson, L., 1889. Laboratory notes of technical methods for the nervous system. *N. Y. Med. J.* 50, 57–60.
- Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* 231, 232–235.
- Weigert, K., 1904. Eine kleine Verbesserung der Hämatoxylin van-Gieson-methode. *Ztschr. wiss. Mikr.* 21, 1–5.
- Yip, L., Dart, R.C., Gabow, P.A., 1994. Concepts and controversies in salicylate toxicity. *Emerg. Med. Clin. N. Am.* 12, 351–364.