Effect of Formaldehyde on Rat Liver in Doses Used in Pulpotomies

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Many studies have questioned the toxic effects of formocresol, one of which is its systemic distribution. This study focused on determining whether there was risk of acute hepatic lesion after the use of intravenous formaldehyde in doses for multiple pulpotomies in rats. Histological and biochemical changes were evaluated. Results showed that very high doses of formaldehyde injected into rats, doses that were much higher than those given for multiple pulp treatments in a single session in Pediatric Dentistry, showed no signs of liver toxicity.

***Key Words**: formocresol, formaldehyde, pulpotomy, liver toxicity, systemic distribution*
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INTRODUCTION

Pulpotomies in primary teeth continues to be one of the most common treatments in Pediatric Dentistry. The word pulpotomy refers to the surgical removal of the coronal portion of the affected dental pulp and the subsequent treatment of the radicular pulp to conserve its function and vitality.[1,2] Pulpotomy is classified in accord with the following therapy objectives: devitalization (mummification, cauterization), preservation (minimum devitalization) or regeneration (repair). Devitalization refers to fixation of vital tissue and is characterized by use of formocresol. Preservation indicates an attempt to maintain the maximum amount of vital tissue as occurs with ferric sulphate and, in its day, glutaraldehyde. Finally, the most important objective of regeneration is to maintain vital tissue and stimulate reparative dentin formation.[3,4]

However, the most widespread treatment has been with formocresol. The most common formula was introduced by Buckley at the start of the XXth century and consists of 19% formaldehyde, 35% cresol, 15% glycerine and water.[5,6] The active ingredients are formaldehyde, as a fixing agent, and cresol, which permits distribution. Glycerine is used as an emulsion and to prevent formaldehyde polymerization. The technique of formocresol pulpotomy has undergone several changes: from its application in several sessions at full concentration in order to fully fix the pulp (Sweet, 1930), to a single 5-minute application (Berger, 1965) and subsequently its use at a concentration at 20% of the original formula (Morawa, 1975).[3, 4, 7-9]

Despite using 20% formocresol for only 5 minutes, clinical and radiological success is very high. Depending on the author, it ranges from 70-97%, which supports the technique's use and means it is still the standard technique for pulpotomy in primary teeth and the bench-mark for comparison with other agents.[10-17]

However, in many of the studies conducted, the use of formocresol is widely questioned, on account of its undesirable effects, i.e. its local and systemic toxicity and its mutagenic, carcinogenic and immunogenic potential.[3,18-24] Although systemic toxicity has not been studied in humans, animal research has shown that 14C-marked formaldehyde molecules, once formocresol pulpotomy has been performed, are distributed through the pulp and dentin, crossing the periodontal ligament.

Pashley *et al.*[26] studied the distribution of the ^{14}C-formaldehyde contained in the formocresol delivered to the pulp chamber in pulpotomy. The highest plasma levels were reached thirty minutes after pulpotomy. A small part of the absorbed dose is metabolized and is eliminated as $^{14}CO_2$. Most of the absorbed dose is found in tissues, especially the liver and biliary system.

Myers *et al.*[27] demonstrated tissue changes in liver and kidney that suggested acute toxicity, after 16 pulpotomies were conducted on a dog in a single session. The animal was sacrificed after six hours and a reduction in the width of Bowman's space could be seen as a result of edema in the glomerular capillaries and an edema in hepatic tissue that changed the appearance of the hepatic sinusoids.

In a study of rats, Ranly[28] calculated the systemic distribution of formaldehyde as 30% of the dose delivered to the pulp chamber. It was accepted that, after formocresol pulpotomy, there is systemic distribution of formaldehyde.

The current study focused on determining whether there is a risk of acute hepatic lesion after the use of formocresol in pulpotomies. The objective was to analyze systemic toxicity at therapeutic doses in formocresol pulpotomies, by evaluating the histological and biochemical changes, i.e. the levels of glutamic-oxaloacetic and glutamic-pyruvic (GOT-AT and GPT-ALT) transaminases after the delivery of intravenous formaldehyde.

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METHODS AND MATERIALS

A sample of 32 male Sprague Dawley rats weighing about 200 gr. was used. The sample was divided into four groups of eight rats. Since, in Sprague Dawley rats, pulpotomy is only possible in two upper molars, a situation simulating that of the patient who has several pulpotomies done at the same time while he/she is under sedation or general anesthesia cannot be reproduced. Therefore, it was decided to study the systemic effect of formaldehyde by means of intravenous injection of a dose equivalent to the dose distributed systemically after pulpotomy. The Ethics Committee on Animal Experimentation approved this study, as it complies with the regulatory guidelines An identical volume of physiological serum was delivered to the first group (control) and the other three groups. In groups two, three and four, formaldehyde was delivered intravenously in amounts equivalent to the amount distributed systemically after ten pulpotomies in group two, twenty pulpotomies in group three and one hundred pulpotomies in group four.

Group 1: Control	Intravenous injection of physiological serum	0.25 ml
Group 2	Intravenous injection of formaldehyde equivalent to 10 pulpotomies	3.8 µmol
Group 3	Intravenous injection of formaldehyde equivalent to 20 pulpotomies	7.6 µmol
Group 4	Intravenous injection of formaldehyde equivalent to 100 pulpotomies	38 µmol

The formaldehyde doses delivered followed the amounts in the findings of Ranly[28], who showed by means of ^{14}C-marked formaldehyde the systemic distribution of formaldehyde at 30% of the dose delivered to the pulp chamber.

Ranly *et al.*[29] used in the pulp chamber of the rat molar 0.2 µl of formaldehyde solution at 19%, equivalent to 1.26 µmol of formaldehyde, of which 0.38 µmol were distributed systemically through each of the molars treated.

Then, a 19% formaldehyde solution was used to prepare dilutions for delivering 3.8 µmol of formaldehyde in group two, 7.6 µmol in group three and 38 µmol in group four.

All rats were anesthetized by intraperitoneal injection of Ketamine (30 mg / kg) and diazepam (4 mg / kg). Fifteen minutes before injecting the anesthetic, they were given atropine by intramuscular pathway (0.05 mg / kg).

Formaldehyde was delivered through the caudal vein of the rat after aspiration to check that injection really was into the vein.

12 and 24 hours after the start of the experiment, blood samples from the caudal vein of all the animals were taken for analysis.

After collection of the last blood samples, all rats were killed by an overdose of anesthetic. Samples of hepatic tissue were taken for subsequent histological analysis.

For biochemical analysis, the levels of glutamic-oxaloacetic transaminase GOT-AST (aspartateaminotransferase) and glutamic-pyruvic transaminase GPT-ALT (alaninaminotransferase) were determined in the blood samples collected 12 and 24 hours after the intravenous delivery of formaldehyde. The blood samples were collected in microtainers and were then centrifuged to separate the plasma. Finally, a HITACHI 917 auto-analyzer was used for the biochemical tests.

The biochemical results were analysed with the SPSS computer program. The Levene test checked the homogeneity of the variance of each variable, since this is a necessary precondition to the ANOVA parameter test. 95% significance level was used.

RESULTS

The histological examination of hepatic tissue was practiced by the same anatomo-pathologist, who was ignorant of the groups to which the samples belonged. The pathologist was asked to look for signs of cell necrosis in the samples analyzed and define them as normal or abnormal, verified by a qualitative study.

None of the samples studied had noteworthy changes; nor were differences found between the samples of the different groups studied. It should be emphasized that, on magnifying the histological cuts, the hepatocytes were found to be structurally undamaged, with no signs of inflammatory filtration or necrosis, as might be expected after the delivery of a hepatotoxic product.

Figure 3 shows one of the samples of hepatic tissue from the rats that were delivered a formaldehyde dose equivalent to the amount distributed systemically after 100 pulpotomies. The characteristics of the hepatic parenchyma were identical to those of the rats belonging to the control group.

The biochemical analysis consisted of measuring the activity of ALT and AST (Tables 1 and 2). Readings were taken 12 and 24 hours after delivery of the formaldehyde dose and results showed there were no significant differences ($p>0.01$) in the four groups studied between AST and ALT values at 12 and 24 hours. In addition, in each group no significant differences were found between the values at 12 and at 24 hours. The significance of the test was very close to the pre-established a value (0.01) only in the 100-pulpotomies group.

DISCUSSION

The formocresol pulpotomy technique has been used in Pediatric Dentistry since 1930. Formaldehyde, a well-known toxic product, is one of the ingredients of formocresol. Its use in dentistry was formerly considered risk-free, since its action was thought to be restricted to pulp tissue.

Recently, in the European Union, commercial dental products containing formaldehyde have been withdrawn from the drugs market, a restriction which, however, does not exist in the United States. Pediatric Dentistry is now confronting the dilemma of whether to continue using formocresol, which has very high success rates but entails a "presumed" risk of toxicity, or to replace it with other drugs which are sometimes more complex to use or very expensive and whose success rate in no case exceeds formocresol's.

Since it was impossible to practise multiple pulpotomies on the same animal to evaluate the acute hepatic toxicity of the drug under study, we resorted to intravenous inoculation of a dose of the drug, established on the basis of the findings of studies showing the systemic distribution of formaldehyde.

The formaldehyde doses distributed systemically at each pulpotomy were taken from the study by Ranly,[28] who calculated it at 30% of the dose delivered to the pulp chamber. We believe this figure is very high, when compared for example with the figure found by Myers,[25] but it serves the objectives of the experiment better. The values are higher than those found by other authors in experiments

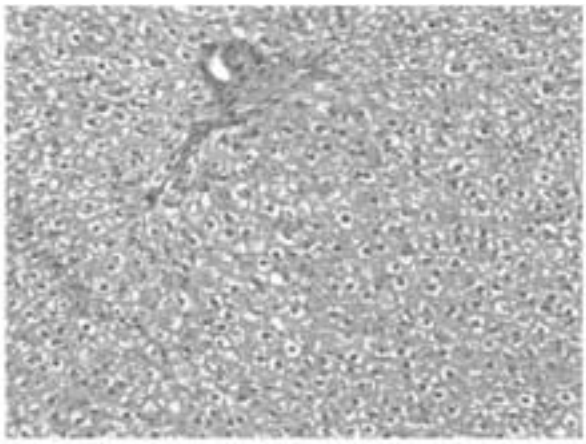

Figure 3: Group with a dose equivalent to 100 pulpotomies; hepatic parenchyma

on monkeys and dogs,[25,26] but Ranly attributed this to the differences in pulp anatomy between rats and other mammals, to the delivery of formaldehyde with a pipette instead of a cotton swab, to the delivery of formaldehyde rather than formocresol and to the different metabolic rate of rodents. Ranly's studies[28] focused on formaldehyde, as it is the element in formocresol related to possible toxicity, mutagenicity and carcinogenesis. Cresol is not very soluble in a watery medium, which greatly limits its absorption during the period of formocresol delivery to the pulp chamber.

Table 1

AST	N	12 h.		24 h.	
		Mean	Standard deviation	Mean	Standard deviation
Control group	8	81.55	7.15	82.17	7.33
10 Pulpotomies	8	82.70	20.22	89.68	49.73
20 Pulpotomies	8	76.03	27.19	101.45	84.68
100 Pulpotomies	8	87.27	31.17	76.33	34.86
Total	32	81.89	22.51	87.41	50.55

Table 2

ALT	N	12 h.		24 h.	
		Mean	Standard deviation	Mean	Standard deviation
Control group	8	39.31	5.27	41.45	6.12
10 Pulpotomies	8	32.94	8.07	31.19	11.67
20 Pulpotomies	8	39.29	14.25	39.44	8.38
100 Pulpotomies	8	43.49	8.35	32.16	7.33
Total	32	38.75	9.86	36.06	9.36

The drug was inoculated into the caudal vein of the rat, despite a certain difficulty due to its narrowness. Other inoculation pathways, such as into the jugular vein after its surgical exposure, as Ranly[28] describes, were discarded as they were thought very traumatic for the animal and might increase mortality before the conclusion of the experiment due to the risk of infection in the incision. In fact, Ranly described in his study patterns of respiratory stress and irritability in some experimental animals, which might be explained by the surgical trauma to which they were subjected.

In our study, histological examination of hepatic tissue showed no anomaly suggesting liver cell lesion. Results were similar in all groups, with no differences between the control and the groups injected with various doses of formaldehyde.

The only description of histological changes as a consequence of formocresol pulpotomy are found in Myers' study.[27] In only one of the five dogs studied after sixteen pulpotomies did Myers find any reduction in the width of the sinusoids due to edema and inflammation of hepatocytes, along with a certain uneven staining in the cells around the central vein of the lobule. It is clear that the procedure used by Myers[27] permitted better comparison with humans. However, this study conducted no biochemical tests, and these in our view are more sensitive to determining the existence of a hepatic cell lesion, even though the lesion might be reversible.

Ranly *et al.*[29] also analyzed the histological changes caused in hepatic tissue by the intravenous delivery of formaldehyde and found no noteworthy anomalies under the microscope, either. The Ranly study used rats, with the biggest dose delivered equivalent to the amount distributed after 250 pulpotomies.

Our biochemical analysis gave findings that were consistent with those of Ranly and cols.[29], who only analyzed glutamic-oxaloacetic transaminase and found figures that did not greatly differ from control group figures after delivery of a formaldehyde dose equivalent to 250 pulpotomies. Glutamic-pyruvic transaminase was not determined in this Ranly study.

Nevertheless, when new treatment options to replace formocresol pulpotomy are proposed in the literature, reference is usually made to Myers' histological findings[27] after sixteen pulpotomies on a dog (discussed above). Performing several pulpotomies in a single treatment session is customary in young patients with early childhood caries, who need to be sedated or anesthetized during treatment. In these cases the formaldehyde that is distributed systemically is greater, depending on the number of pulpotomies. The dilution of the formaldehyde absorbed depends on body mass, which is low in young patients, who are usually candidates for treatment under general anesthesia. The immaturity of the organs controlling metabolism and excretion may also cause higher-than-expected formaldehyde levels in these young patients.

Given the lack of studies confirming the acute toxicity results described by Myers,[27] our study falls into line alongside those of Ranly[28,29], in which no histological or biochemical changes were found after the delivery of doses considerably higher than those that might penetrate the circulatory system after pulpotomies. Our study expands the examination of hepatic enzymes and modifies the delivery pathway in order to reduce the post-operation trauma caused by cutting the jugular vein. We believe that future studies could monitor several biochemical parameters of liver and kidney function in patients undergoing multiple pulp treatments under general anesthetia.

It is clear that a study of rats cannot demonstrate conclusively that formocresol is absolutely innocuous for our patients, but it can serve

as a starting-point for studies of animals that are much closer to humans, such as dogs or monkeys.

In addition, in the studies referring to the systemic distribution of formaldehyde, there is no reference to the characteristics of the molecule to which the radioactive carbon belongs. The nature of this molecule us what enables formaldehyde, metabolized in pulp or periapical tissue and transformed into CO_2, to be distributed in the blood stream. We assumed that measurements of tissue radioactivity corresponded to the presence of formaldehyde in these tissues and that the distribution percentages are as found in the literature.

Despite all this, prudence should govern our actions, especially when we are working with young children whose drug-metabolizing capacity is not always comparable to an adult's or a school-age child's. As such, it is advisable to use diluted formaldehyde solutions (20%) and not the solution described initially by Buckley, and to employ formocresol in the pulp chamber for a shorter period of time. These precautions will be more strongly indicated for those patients who receive many treatments in a single session and who, because of their youth and low weight, absorb a proportionally much greater dose, which in unfortunate circumstances could trigger histological changes in liver cells.

CONCLUSIONS

• The histological examination showed no damage of hepatic tissue, with no signs of inflammatory filtration or necrosis in the different samples studied.

• The biochemical study determined that the transaminase levels detected in blood at 12 and 24 hours showed no significant differences between the control group and the remaining groups.

• In the current study, the very high doses of formaldehyde (the main and most questioned ingredient of formocresol) injected in rats, doses that were much higher than those needed for multiple pulp treatments conducted in a single session in Pediatric Dentistry, showed no hepatic toxicity.

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