Evaluation of the mortality rate caused by different periods of selective portal vein occlusion in rats[1]

Avaliação da mortalidade causada por diferentes períodos de oclusão seletiva da veia porta em ratos

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ABSTRACT

Mortality from acute selective portal vein occlusion (SPVO) is a matter of concern for surgeons during the management of traumatic portal vein injury. However, mortality rates related to different periods of SPVO remains undetermined. **Purpose**: To determine the mortality rates resulting from different periods of acute SPVO in rats. **Methods**: Wistar male rats were randomized into 8 experimental, and 8 control groups. Experimental animals underwent SPVO during 15 to 75 minutes, and control groups underwent sham procedures. All surviving animals were followed up to 14 days for assessment of mortality rate. **Results**: Death rates varied from 0% in the 15 min SPVO group, to 100% with 65 and 75 minutes of SPVO. A strongly positive correlation was observed between mortality rates and SPVO periods ($p<0.001$) with either linear or quadratic regression analysis tests. All deaths in the 20min and 25min SPVO groups occurred after 75 min from the moment of clamping (or after 60 min from unclamping); death from 30 or more min SPVO occurred predominantly within 75 min from clamping moment (or within 60 min from unclamping). (Exact Fisher test, $p=0.009$). **Conclusions**: The mortality from SPVO in rats increases with longer duration of SPVO; with deaths occurring later for short periods (≤ 25 min) of SPVO and earlier for longer periods (≥ 30 min) of SPVO.

Key words: Portal Vein. Constriction. Mortality. Rats.

RESUMO

A mortalidade da oclusão seletiva da veia porta (OSVP) preocupa os cirurgiões durante o tratamento de lesão traumática da veia porta. Entretanto, as taxas de mortalidade decorrentes de diferentes períodos de OSVP não estão determinadas. **Objetivo**: Determinar a mortalidade Decorrente de diferentes períodos de oclusão seletiva da veia porta (OSVP) em ratos. **Métodos**: Ratos Wistar machos foram randomizados em 8 grupos experimentais e 8 controles. Os experimentais foram submetidos a OSVP por períodos de 15 a 75 minutos, seguidos de observação até o óbito, e os sobreviventes até 14 dias. Os grupos controles receberam idênticos procedimentos, exceto a OSVP. **Resultados**: A mortalidade, a partir do momento da oclusão, aumentou progressivamente, de 0% no grupo de 15min, atingindo 100% nos grupos de 65min e 75min de OSVP. Houve alta correlação positiva entre mortalidade e duração da OSVP ($p<0.001$; tanto em teste de Regressão linear quanto quadrática). Os óbitos decorrentes de 20 e 25 min de OSVP ocorreram após 75min do clampeamento (ou após 60 min do desclampeamento); os decorrentes de 30min ou mais ocorreram predominantemente antes de decorridos 75 min do clampeamento (ou 60 min do desclampeamento). (Teste exato de Fisher, $p<0.05$). **Conclusões**: A mortalidade da OSVP em ratos aumenta com a maior duração da oclusão; os óbitos ocorrem mais tardiamente após oclusões breves (≤ 25 min), e mais precocemente nas oclusões mais prolongadas (≥ 30 minutos).

Descritores: Veia Porta. Constrição. Mortalidade. Ratos.

Introduction

Mortality resulting from portal vein occlusion has long been investigated[1-3] As a surgical procedure, portal vein occlusion may be achieved by means of the portal triad (common bile duct, hepatic artery, and portal vein) occlusion (PTO), or by means of clamping the portal vein after its isolation from the portal triad, allowing the selective portal vein occlusion (SPVO). Portal triad occlusion (PTO) was first performed by Pringle in 1908, in patients sustaining traumatic liver injury, and became known also as the Pringle's maneuver,[4] that is frequently employed in the surgical management of traumatic liver injuries, and in selective hepatectomies. Studies in rats subjected to PTO for 30 minutes[5] or to 45 minutes,[6] without mortality, were reported. However, a 9% mortality following 30 minutes of PTO in rats has also been reported.[3] In an experimental rat model of PTO, circulatory, hemodynamic, and metabolic disturbances, as well as mortality rates, have been determined after 5 to 60 minutes of PTO, and although no mortality was observed with 5 to 45 minutes of PTO, only one of the 8 animals with 60 minutes of PTO survived.[6] In humans, 30 minutes of PTO has been accepted as a safe procedure, and is usually employed in liver surgery.[7] More recently, Fontelles et al[8] demonstrated a clear correlation among hemodynamic and metabolic variables in rats subjected to hemorrhagic shock followed by 15 minutes of PTO; although not focused on mortality, the study points out the continued relevance of PTO in the surgery of trauma. On the other hand, selective portal vein occlusion (SPVO) may be achieved by means or ligation of the vein[1]. Boyce performed transient SPVO in dogs, and observed that occlusion periods of 33 to 134 (average 87) minutes resulted in the death of all the 7 animals tested, leading to the conclusion that complete occlusion of portal vein is incompatible with life[2]. Child et al, in 1950 performed the ligation of portal vein first in monkeys, without mortality, and later during surgery for gastric cancer in two patients who died two months later from carcinomatosis[9]. Makino et all 2005, compared the effect of SPVO versus PTO on the recurrence-free survival time after hepatectomy in patients with hepatocellular carcinoma, and concluded that SPVO was associated with better disease-fee survival[10]. A more recent study discussed the safety and the feasibility of the use of laparoscopic selective portal inflow occlusion in seven patients undergoing hepatic segmentectomy or hemihepatectomy, and concluded that laparoscopic SPVO is a safe and feasible technique for this procedure[11]. Although in the management of traumatic liver injuries, and also in elective hepatectomies, transient portal vein occlusion is usually accomplished by means of Pringle's maneuver (PTO), the management of traumatic injuries to the portal vein in emergency surgery usually requires transient SPVO that is achieved by means of clamping the isolated portal vein. In rare occasions, in the management of severely shocked trauma patients with severe bleeding injury to the portal vein, ligation of this vein may be employed as the only alternative maneuver to save the patient's life[12]. In a study of 15 patients with traumatic injury to the portal vein, Pearl et al discussed the most effective procedures for the management of this injury, and reported 14% mortality for venorrhaphy, and 33% for portal vein ligation[13]. Thus, although traumatic injuries to the portal vein are rare, they are associated with high mortality, and the most effective management of portal vein injury remains undetermined. It is usually accepted that human liver can tolerate warm ischemia for at least 60 minutes. However, a recent observation in two cases of liver transplantation has shown that a short term portal vein occlusion lead to acute steatosis in the liver, a fact that was not previously reported, and its mechanism remains unclear. Although the authors did not specify the duration of the short period of portal vein occlusion, this finding points out a new adverse effect of portal vein oclusion. Studies on the possible mechanisms of disturbances resulting from portal vein occlusion have suggested that total portal vein occlusion may induce intestinal congestion leading to partial intestinal ischemia, with blood flow reduction beyond the compensatory increased oxygen extraction. These changes could allow the occurrence of intestinal mucosal injuries, increased mucosal permeability, intestinal barrier damage, endotoxin/bacterial translocation, which may lead to the development of sepsis and multiple organ system failure[15,16]. Thus, although different studies have focused on the mechanisms of the adverse effects of portal vein occlusion in experimental animal models, the mortality rate resulting from diverse periods of SPVO clamping has not been clearly determined. Therefore the objective of this study was to evaluate the mortality rate by different periods of selective occlusion of portal vein in rats.

Methods

The study protocol was approved by the Ethics Committee for animal use in research studies of the institution where the research was performed. Ninety Wistar male rats weighing from 220 to 327g (278.5 ± 22.6g) were used in this study. Eight different SPVO periods (15, 20, 25, 30, 45, 60, 65, and 75 minutes) (n=10) were studied. For the experimental group with the longest period (75 minutes) of portal vein occlusion, the animals were randomized into this group (n=10) and the control (CTR) group (n=10). All the animals of the study were maintained under standard conditions, receiving water and rodent chow *ad libitum*. For the surgical procedures, the SPVO group animals were anesthetized with an intraperitoneal injection of ketamine

chloride (80mg/kg) and xylazine chloride (3.2 mg/kg), and the anterior abdominal wall was shaved and decontaminated with polivynil povidone iodine. A midline abdominal incision was then performed, the hepatic hilum was identified, and the portal vein was isolated from the bile duct and hepatic artery. The portal vein occlusion was achieved by locating a non-traumatic microsurgical clamp (*Vascu-statt*, number 1001-531, Scalan Internat, Minn, 55107, USA), next to the bifurcation of the vein. During the period of SPVO, the abdomen was covered with a plastic wrap in order to prevent water loss by evaporation from abdominal viscera. After the predetermined occlusion period, the clamp was removed, and the abdomen was closed with two layers of continuous monofilament nylon sutures. Rats were then placed in appropriate cages, with free access to food and water, for a 14 days observation period in order to evaluate the mortality rate. Sham-operated (CTR) animals underwent the same procedure described for the corresponding (75 min) SPVO experimental group, except that the portal vein clamping was not performed. These CTR group animals were followed for the 75 min corresponding to the SPVO period of the matched experimental group, and then until 14 days of the experiment. After the observation period, the surviving animals were anaesthetized as described above, and sacrificed through exsanguinations provoked by sectioning the abdominal aorta.

Statistical analysis

Data are expressed as mean values ± SD. Linear and quadratic regression analysis, and Fisher's exact T-test of independent means were performed to evaluate the influences of occlusion periods on mortality of the animals. *P* values < 0.05 were considered significant.

Results

No significant difference in animal weight was found between control and occlusion groups (275.1 ± 25.0g and 275.7 ± 20.1g respectively; $p = 0.98$). The isolated analysis of the occlusion groups showed no statistical difference between weight means of the animals that died and the animals that survived in the different groups (274.0 ± 28.0g and 278.1 ± 20.6g respectively; $p = 0.45$),

Mortality percentages

No animal death was observed in the control (CTR) group, nor in the 15 min of SPVO group. In the experimental groups**,** as the duration of the SPVO period increased beyond 15 min, the number of deaths increased from 10% after 20 min to 90% after 60 min of SPVO. For occlusion periods of 65 min or longer, all tested animals died (Table 1 and Figure 1). All deaths in the SPVO groups occurred less than 24 hours after the moment the PV was clamped, with no death between 24 hours and 14 days. In the SPVO groups, the linear (corrected $R^2 = 0.89$; $p = 0.0001$), as well as the quadratic (corrected $R^2 = 0.96$; $p = 0.001$) regression analysis of the data revealed a significant positive correlation between mortality rates and duration of SPVO periods, according to the equation: $y = 0.01x - 0.08$, where x represents the SPVO period. The analysis of death distribution along the observation period from the moment of portal vein clamping, revealed that deaths caused by 20 and 25 min of SPVO occurred after 75 minutes from the clamping moment, whereas deaths resulting from SPVO periods of 30 min or longer occurred predominantly within the first 75 min following the moment of PV clamping. Exact Fisher test. P=0.009. Considering the deaths occurred following the moment of portal vein unclamping (it is, during the reperfusion period), it was observed that deaths resulting from SPVO periods of 20 and 25 min occurred after the 60 min following the unclamping moment, whereas deaths resulting from SPVO periods of 30 min or longer occurred predominantly within the 60 min following the moment of PV unclamping. Exact Fisher test. P=0.022. Overall, the deaths induced by short term PV occlusion occurred significantly later than those resulting from longer periods of PV occlusion, these occurring predominantly before the end of the first hour of reperfusion (Table 2).

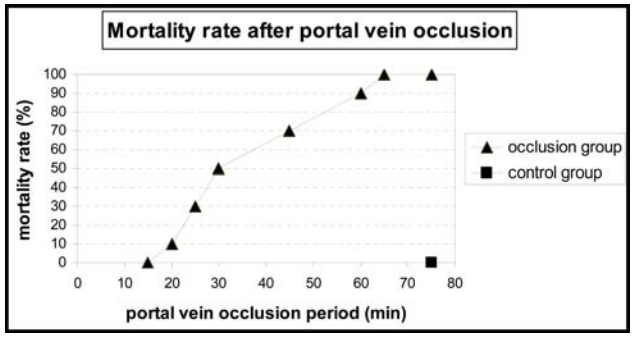

FIGURE 1 - Mortality rate increased from zero at 15 min, till 100% at 65 min of portal vein occlusion (SPVO) periods, and correlated with the duration of SPVO periods. Statistics: Equation: $y = 0.01x - 0.08$ (x = clamping time) Linear correlation: corrected R2 = 0.89; $p = 0.0001$. Quadratic correlation: corrected R2 = 0.96; $p = 0.001$. No deaths occurred in the control group. SPVO: selective portal vein occlusion.

TABLE 1 - Number of deaths observed following the moment of clamping is shown for different periods of SPVO from 15 min (no mortality) to 75 min (100% mortality). Death caused by 20 and 25 min of SPVO occurred after 75 min following the clamping moment, whereas deaths resulting from SPVO periods of 30 min or longer occurred predominantly during the 75 min following the moment of PV clamping. Exact Fisher test. P=0.009. SPVO: selective portal vein occlusion, PV: portal vein

MORTALITY FROM SPVO IN RATS							
Occlusion periods/Study groups	**Deaths observed along 24 hours following the moment of portal vein clamping**					**Total mortality**	
	0 to 30 min	**30 to 60 min**	**60 to 75 min**	**75 to 90 min**	**90 min to 24 hours**	**Nr.**	**%**
15							0
20					1	1	10
25					3	3	30
30	1	2			2	5	50
45		3	2		2	7	70
60		2	2		5	9	90
65		1	8	1		10	100
75		1	8	1		10	100
ALL GROUPS	1	9	20	2	13	45	

TABLE 2 - Deaths resulting from periods of 20 and 25 min of SPVO occurred after 60 min following the unclamping moment, whereas deaths resulting from SPVO periods of 30 min or longer occurred predominantly during the 60 min following the moment of PV unclamping. Exact Fisher test. P=0.022. PV: portal vein, SPVO: selective portal vein occlusion

MORTALITY FROM SPVO IN RATS					
Occlusion periods/Study groups	**Deaths observed Total mortality**				
	During PV occlusion periods	**Within 60 min after the moment of PV unclamping**	**From 60 min of PV unclamping to 24 hours from the moment of PV clamping**	**Nr.**	**%**
15					0
20			1	1	10
25			3	3	30
30	1	2	2	5	50
45		5	2	7	70
60	2	2	5	9	90
65	1	9		10	100
75	9	1		10	100
All groups	13	19	13	45	

Discussion

The duration of portal vein occlusion is a matter of concern for trauma surgeons and has motivated experimental studies employing different periods of selective portal vein occlusion. Filipponi et al.[17] in a report of the benefits of different therapeutic strategies to reduce mortality from portal vein occlusion, employed 45 and 60 minutes of portal vein occlusion in rats. The authors reported that all the animals of the groups that were not subjected to treatment, died within less than 31 hours after PV unclamping[17]. This 100% mortality rate observed by these authors may seem higher than our 70% and 90% observed, respectively for the portal vein occlusion with 45 or 60 min of portal vein occlusion periods. However, the comparison between the two studies may take into account some methodological differences between the two studies. For instance, the

authors used Sprague-Dawlay rats, under ether anesthesia, and we employed Wistar rats, anesthetized with an intraperitoneal injection of ketamine chloride (80mg/kg) and xylazine chloride (3.2 mg/kg). The potential influence of such differences on mortality rate of the animals may be difficult to be evaluated. Although in conditions quite different from those of the present study, Urata et al.[18] investigated the effect of portal vein clamping periods on the survival of rats subjected to orthotopic liver transplantation, and observed that 14 to 17 minutes of portal vein occlusion did not affect animals mortality after the transplantation procedures, while increased occlusion times (18 to 21 min, and 23 to 24 min) were associated with increased mortality[18]. However, in the reviewed literature, neither the correlation of increased duration of SPVO with the increase in the associated mortality rate, nor the longest length of portal vein occlusion period compatible with survival of the animals, have been clearly determined. In the present study, the correlation of SPVO periods with the resulting mortality was evaluated in rats. Initially, five different periods (15, 30, 45, 60, 75) of SPVO, with 15 minutes interval, were employed. However, given the high increase (from 0% to 50%) in mortality rate observed from 15 to 30 minutes of SPVO, two other groups (20min and 25min of SPVO) were added. Likewise, in order to more accurately determine the longer SPVO period compatible with animals survival, the 65 min group was added. Overall, our results indicate a positive correlation between the duration of SPVO periods and mortality rate of the animals (Figure 1). The possible influence of animal age (estimated by the animal weight) was here analyzed for a possible correlation between mortality and animal weight, and no influence of animal age on mortality was observed. In this study, it was observed that all deaths occurred in the SPVO groups within 24 hours, mostly within the 75 min following the moment the portal vein was clamped. Further, it was observed that for SPVO periods shorter than 30 minutes, all death occurred from 75 min to 24 hours following the moment of the portal vein clamping, and that for the longer periods of SPVO employed, most death occurred within these 75 minutes. Considering that some deaths occurred within the longer clamping periods, an analysis was done considering only the deaths occurred during the reperfusion periods, and the results showed that death resulting from 25 and 30 min of SPVO occurred after the first 60 min of reperfusion, whereas deaths resulting from 30 min or longer periods of SPVO occurred predominantly within the first 60 minutes of reperfusion. Although the mechanism of death was not investigated in the present study, our finding that increasing mortality occurs with longer periods of SPVO, and that longer duration of SPVO associates with earlier deaths, may lead to the discussion of the role of hemodynamic, metabolic, and inflammatory mediators on the mechanism of death following SPVO. In the decade 1950, Child observed that portal vein occlusion leads to immediate drop in blood pressure of 30 mmHg and an immediate increase in portal venous pressure to 35 cm saline solution.[9] The possible role of hemodinamic factor as de main determinant of death resulting from SPVO was suggested by Filipponi et al.[17] in a study in rats. The complex interaction of hemodinamic, metabolic, and inflammatory mediators was discussed by Gonce et al.[6] in a study of circulatory and metabolic shock following portal triad occlusion, where a combination of the effects of portal vein and hepatic artery occlusion occurs simultaneously. However, the real mechanism and the moment of death resulting from different periods of selective portal vein occlusion is not clearly determined, and warrants further investigation.

Conclusions

The results of the present study indicate that 15 minutes of SPVO is not a lethal insult in this rat model, and that the mortality rate increases in correlation with the duration of occlusion for periods longer than 15 minutes, achieving 100% of the animals for periods of 65 or more minutes. These results indicate also that death induced by short periods (≤25) minutes of SPVO occurs later than death induced by longer periods (≥30 minutes). More studies are warranted to better clarify the mechanisms of death following SPVO.

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Conflict of interest: none
Financial source: none

Received: January 12, 2007
Review: March 14, 2007
Accepted: April 10, 2007

How to cite this article
Kato LT, Poggetti RS, Fontes B, Massarollo PCB, Younes RN, Heimbecker AMLC, Biroloni D. Evaluation of the mortality rate caused by different periods of selective portal vein occlusion in rats. Acta Cir Bras. [serial on the Internet] 2007 July-Aug;22(4). Available from URL: http://www.scielo.br/acb