Biomedical Research 2016; 27 (3): 860-866

ISSN 0970-938X
www.biomedres.info

Application of solid Carbon dioxide as a novel hemostatic agent on a hepatectomy model in rats.

Ibrahim Ali Ozemir[1*], Cagri Bilgic[1], Erman Aytac[2], Sinan Aslan[1], Baris Bayraktar[1], Banu Isbilen[3], Ebru Zemheri[4], Duygu Kösemetin[4], Rafet Yigitbasi[1], Orhan Alimoglu[1]

[1]Department of General Surgery, Goztepe Education and Research Hospital, Istanbul Medeniyet University, Istanbul, Turkey

[2]Department of General Surgery, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

[3]Department of Biochemistery , Goztepe Education and Research Hospital, Istanbul Medeniyet University, Istanbul, Turkey

[4]Department of Pathology, Goztepe Education and Research Hospital, Istanbul Medeniyet University, Istanbul, Turkey

Abstract

Background: Blood loss and the prolonged operative time for haemostasis is still a great problem after injury or during elective surgery of the liver. Various topical hemostatic agents were introduced to reduce the blood loss. The aim of this study is to evaluate the afficacy of solid carbon dioxide ($SoCO_2$) as a hemostatic agent on hepatectomy model.

Materials and Methods: Thirty-two Wistar albino female rats were included in this study. They were divided in four groups of 8 rats. Haematocrit levels were determined preoperatively. Non-anatomic hepatectomy was performed to the left lobe. Hemostasis was provided with gauze tampons in Group-1, with surgical sutures in Group-2 and with asistance of $SoCO_2$ in Group-3. Hemostasis time and blood loss were recorded. Serum haematocrit, liver function tests were determined postoperatively. $SoCO_2$ assisted hemostasis was provided and relaparotomy was performed after 7 days to determine the late effects of $SoCO_2$ in Group-4.

Results: Blood loss in Group-1 and Group-2 were statistically significant higher than the Group-3 (119.1 ± 22,4 mg; 80.6 ± 18.9 mg; 36.3 ± 11.1 mg, respectively) ($p<0.01$). Therewithal hemostasis time in Group-3 was statistically significant shorter than Group-1 and Group-2 ($p<0.01$). Serum ALT and AST values were increased in first three groups, but did not reveal significant difference between groups (p: 0.045 and p:0.163). ALT and AST values were significantly decreased in Group-4 compared to other groups (p:0.001 and p:0.005, respectively).

Conclusions: $SoCO_2$ assisted hemostasis provide bloodless surgical site, reduced blood loss and also shortened hemostasis time. Although further studies are requires, this study reveals that hepatectomies can be performed safer with $SoCO_2$ assistance.

Keywords: Hemostasis, Solid carbon dioxide, Hepatectomy.

Accepted on March 31, 2016

Introduction

Sinusoidal structure of liver parenchyma lacks smooth muscle fibers providing vasoconstriction. Thus, when hepatic tissue integrity is distrupted, hard-to-control, significant bleeding is noted [1]. Mortality secondary to liver traumas is 10-15%, the most important factor being bleeding [2,3]. Likewise, in elective surgical resections performed for primary or metastatic liver tumor, bleeding and its secondary complications, continue to be one of the most important trouble [4,5]. In order to perform liver surgery in a safer fashion and to shorten hemostasis time, bipolar and monopolar electro-cauters, ultrasonic dissectors, radiofrequency equipments and various hemostatic agents are utilized [6].

In cryosurgery, the purpose is to produce vascular stasis and microcirculation defect by exposing the tissue to freezing and liquefaction temperatures [7,8]. In recent years, Argon (-186°C) and Nitrous oxide (-89.5°C) are used as cooling agents in cryosurgery. Response of tissues to cold differs according to degree, duration of exposure and tissue type [9]. Mildly freezing short-term cold exposure produces inflammatory response; whereas severe freezing cold results in cell necrosis secondary to circulatory disruption [7,8]. Carbon dioxide (CO_2) is a chemical in gaseous state under normal

conditions liquidates under high pressure and solidified under laboratory conditions. Surface temperature of solid carbon dioxide is -78.5°C. It can directly proceed to gaseous from solid state (sublimation). Therefore, not causing carbonization or leaving remnant material on the tissue it comes in contact with having a high cooling capacity (152 Kcal/kg) during sublimation. The aim of this study is to evaluate the hemostatic effect of solid CO_2 ($SoCO_2$), a low-cost, easily available agent, on the hepatectomy model that was generated using rats.

Material and Methods

This study was carried out at Marmara University Animal Experiments and Research Laboratories under the June 17, 2011 no."36.2011. Mar" permit of "Ethical Association of Marmara University Animal Experiments". Thirty-two Wistar albino female adult rats, weighing 250-300 grs were randomly divided into 4 groups of 8. Four rats were placed in each cage, fed ad libitum, and followed in 18-23°C room temperature of 50-55°F humidity for 12 hours/day and 12 hours/night.

Under general anesthesia with ketamin hydrochloride (100 mg/kg) and xylazine hydrochroride (10 mg/kg), blood sample was drawn from caudal vein to determine pre-operative hematocrite levels of rats. Laparotomy was performed via an abdominal midline incision and suspension ligaments of liver were released. Blotting papers were used to calculate the amount of bleeding. Prior to the resection, weight of the blotting papers were weighed on a precision scale and placed under the liver left lobe (Figure 1A). Consequently non-anatomic surgical resection was performed to the left lobe of liver (Figure 1B). The area of the sectional surface of liver were measured using milimetric paper and also weight of resected liver tissue were measured on analytic scale. In Group-1 gauze tampon press was applied to resected surface until hemostasis was achieved (Figure 1C). In Group-2, sectional surface hemostasis was achieved by 5/0 prolene continuous sutures (Figure 1D). In Group-3, sectional surface of liver was contacted with $SoCO_2$ for 20 seconds (Figure 2A). Bleeding was controlled after spontaneous melting of frozen surface (Figure 2B). Re-bleeding from the hepatic vessels was observed after melting. Sectional surface was re-frozen with $SoCO_2$ and tip of the vessels were ligated with 5/0 prolene in a bloodless environment (Figure 2C). At the end of each freezing/melting period, blotting papers were re-weighed to calculate the total bleeding amount (Figure 2D). Hemostasis was observed for 10 minutes in each group, 5cc intracardiac blood sample was drawn to evaluate the blood tests and haematocrit levels. Rats were sacrificed under general anesthesia. Remnant liver tissue was resected and fixated with 10% formaldehyde for pathologic research. Procedure processing time was recorded.

To determine the presence and amount of acute hepatocellular destruction aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma glutamil transferase (GGT), total bilirubin, direct bilirubin, albumin, prothrombin time (PT), INR and haematocrit values were established.

SPSS software version 20.0 (SPSS Inc., Chicago, IL) was used to analyze the data of this study. Kolmogorov-Smirnov distribution test was used for related statistical methods (Frequency, Percentage, Average, and Standard Deviation) and to examine the normal distribution. Quantitative data were compared with Mann Whitney U and Kruskal Wallis tests. Wilcoxon test was used to compare the parameters within the group. Results were reviewed in a confidence interval of 95%, and a value of $p<0.05$ was accepted as statistically significant.

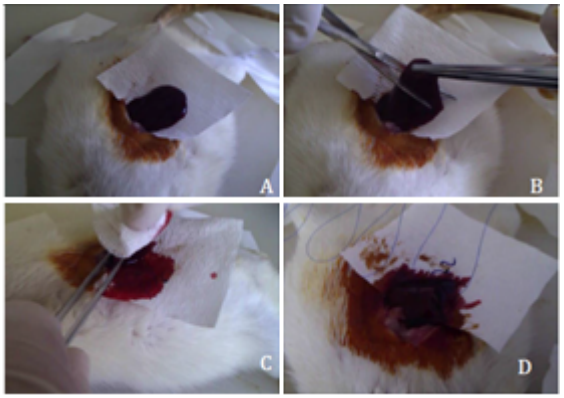

Figure 1. *A- Liver left lob of rat to be resected. B- Nonanatomic resection model. C- Hemostasis with gauze compression in Group-1. D- Suturing the liver sectional surface in Group-2.*

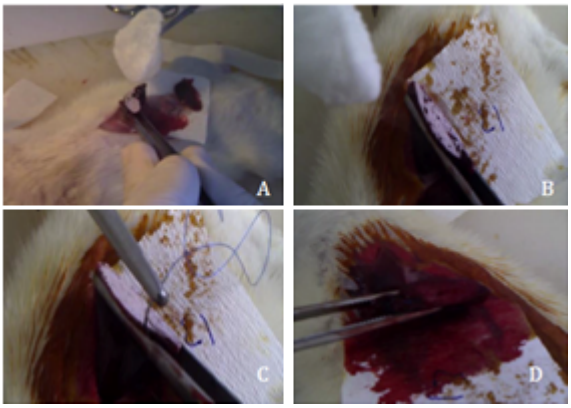

Figure 2. *$SoCO_2$ assisted hemostasis in Group-3 and Group-4. A- Contact of $SoCO_2$ to the liver. B- Appearance of liver sectional surface after contact of $SoCO_2$ and re-bleeding from the larger vessels during melting. C- Suturing the larger vessel regions after re-contact with $SoCO_2$. D- Apperance of sectional surface of liver following hemostasis.*

Homeostasis model used in Group-3, was also used for Group-4. In Group-4 rats were not sacrificed after hemostasis and abdominal incision was closed with 4/0 prolene. They observed for 7 days in unrestricted conditions. At the end of the 7th day, re-laparotomy was performed via abdominal midline under general anesthesia that provided with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). Early stage effects of $SoCO_2$ on the contact surface of liver tissue and the presence of intraabdominal abcess, seroma, biloma or adhesion were observed. Euthanasia was applied after drawing 5cc intracardiac blood.

Results

The mean weight of rats was 234.5 ± 9.3 gr there was no significant difference between the groups with regard to weight of rats (p:0.725).

Table 1. Comparision of experimental and surgical data between groups.

	Group-1 (Mean ± SD)	Group-2 (Mean ± SD)	Group-3 (Mean ± SD)	P vlue
Rat weights (gr)a	234.4 ± 10.5	236.3 ± 8.7	232.5 ± 8.5	0.725
Liver section surface (mm^2)a	74.1 ± 6.2	76.2 ± 10.7	72.1 ± 16.1	0.637
Resected segment weight (mg)a	0.35 ± 0.02	0.34 ± 0.21	0.35 ± 0.06	0.931
Mean blood loss (mg)a	119.1 ± 22.4	80.6 ± 18.9	36.4 ± 11.1	0.001*
Pre-op hematocrita, b	54.2 ± 3.2	52.7 ± 4.9	53 ± 3.7	0.709
Post-op hematocrita,b	47.4 ± 10.1	47.8 ± 4.8	47.7 ± 3.6	0.919
Hemostasis time (sec.)a	377.5 ± 56.7	203.7 ± 69.7	176.2 ± 56.8	0.001*

*: p<0.01

a: Kruskal Wallis test

b: Wilcoxon test

In comparison of groups with regard to weight of resected liver tissue and sectional surface areas, there was no statistically difference (p:0.637, p:0.931, respectively) (Table 1).

Blood samples from caudal vein drawn pre-procedure and intracardiac sample drawn post-procedure were used for determination of hematocrit (Htc) values. No significant pre or post-procedure Htc value difference was found among the groups (p:0.709, p:0.919, respectively). In Group-3 in which hemostasis was achieved with $SoCO_2$ mean bleeding amount was 36.4 ± 11.1 mgr where as it was 119.1 ± 22.4 mgr in Group-1 and 80.6 ± 18.9 mgr in Group-2. Statistical comparison among groups showed that bleeding was significantly less in Group-3 where hemostasis was achieved with $SoCO_2$ (p: 0.001).

The mean $SoCO_2$ contact time was found 38.7 ± 5.8 (30-45 second) to achieve the hemostasis in Group-3 and Group-4. The length of time until hemostasis was also compared between groups. The mean hemostasis time in Group-3 was 176.2 ± 56.8 sec. whereas it was 377.5 ± 56.7 sec. in Group-1 and 203.7 ± 69.7 sec. in Group-2. Statistical comparison of hemostasis time among the groups showed that hemostasis time was significantly shorter in Group-3 compared to Groups 1 and 2 (p:0.001) (Table 1).

Blood samples were studied to determine acute effects of $SoCO_2$ on the liver. Serum ALT, AST and LDH levels were elevated in all groups (Table 2); however there was no statistically significant difference between the groups with respect to serum albumin, total bilirubin, direct bilirubin, ALP, ALT, AST, PT, INR and Htc levels. In Group-3 mean LDH level was 1066.4 ± 561.7 U/L, whereas this level in Group-1 and Group-2 was 1827.3 ± 537.6 U/L and 2074.4 ± 766.1 U/L, respectively. Based on these results, LDH value increase was statistically significant lower in Group-3 where hemostasis was provided with SoCO2 (p: 0.010) (Table 2).

Table 2. Comparison of biochemical data obtained from intracardiac blood samples within groups.

	Group-1 (Mean ± SD)	Group-2 (Mean ±SD)	Group-3 (Mean ±SD)	P value
Albumin (g/dl)a	3.2 ± 0.2	3,6 ± 0,3	3.1 ± 0.2	0.005*
Total bilirubin (mg/dl)a	0.13 ± 0,01	0.16 ± 0.05	0.13 ± 0.06	0.266
Direct bilirubin (mg/dl)a	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.967
ALP(U/L)a	170.1 ± 36.7	155.1 ± 50.9	128.2 ± 39.8	0.090
AST(U/L)a	294.6 ± 46.1	249.4 ± 102.8	273.6 ± 172.9	0,163
ALT(U/L)a	174.2 ± 71.5	93.7 ± 32.8	146.4 ± 68.1	0.045
LDH (U/L)a	1827.3 ± 537.6	2074.4 ± 766.1	1066.4 ± 561.7	0.01*
PT (sn)a	9.5 ± 0.7	9.8 ± 1.6	10.41 ± 1.1	0.158
INRa	0.78 ± 0.01	0.81 ± 0.14	0.84 ± 0.08	0.275

*: p <0.01

a: Kruskal Wallis tests

To examine the possible adverse effects of $SoCO_2$ contact in the early stage, serum ALT, AST, ALP, LDH, PT and INR levels of rats were compared between Group-3 and Group-4 that observed for 1 week after $SoCO_2$ contact. There was no statistically significant difference between Group-3 and Group-4 with respect to weight of rats, sectional surface area of liver, resected tissue amount, mean bleeding amount, pre-operative and post-operative hematocrite values. AST and ALT values that evaluated to estimate hepatocellular damage, were significantly reduced in Group-4 (p:0.005, p:0.001) (Table 3).

ALT values were regressed to normal levels, however LDH and AST values were still remain above the normal limits after a week`s follow-up in Group-4. Group-3 and Group-4, did not show statistically significant difference in albumin, total/direct bilirubin, ALP, LDH, PT and INR values.

Table 3. *Comparison of rats features and biochemical values in group-3 and group-4 which undergo $SoCO_2$ assisted hemostasis.*

	Group-3 (Mean ± SD)	Group-4 (Mean ± SD)	p value
Rat weights (gr)[a]	232.5 ± 8.4	235 ± 10.3	0.591
Liver section surface (mm^2)[a]	72.1 ± 161.1	70 ± 4.5	0.156
Resected segment weight (mg)[a]	0.35 ± 0.06	0.34 ± 0.03	0.561
Mean blood loss (mg)[a]	363.8 ± 11.1	353.7 ± 17.1	0.156
Pre-op hematocrit[a]	53 ± 3.7	54 ± 2.6	0.449
Post-op hematocrit[a]	47.8 ± 3.6	48.5 ± 2.5	0.667
Hemostasis time (sec.)[a]	176.3 ± 56.8	138.1 ± 23.6	0.1
SoCO2 Total contact time (sn)[a]	38.8 ± 5.8	37.5 ± 4.6	0.507
Albumin (g/dl)[a]	3.1 ± 0.2	3.2 ± 0.3	0.431
Total bilirubin (mg/dl)[a]	0.12 ± 0.06	0.16 ± 0.03	0.181
Direct bilirubin (mg/dl)[a]	0.02 ± 0.01	0.02 ± 0.01	0.546
ALP(U/L)[a]	128 ± 39.9	158.5 ± 58.5	0.059
AST(U/L)[a]	273.6 ± 172.9	147.8 ± 19.2	0.005*
ALT(U/L)[a]	146.4 ± 68.1	54 ± 10.9	0.001*
LDH (U/L)[a]	1066.4 ± 561.7	909 ± 418.7	0.753
PT (sn)[a]	10.4 ± 1.12	9.8 ± 0.64	0.225
INR[a]	0.84 ± 0.08	0.80 ± 0.06	0.267

*:p <0.01

[a]: Mann Whitney U tests

Following euthanasia, liver tissue which came in contact with $SoCO_2$ was resected and fixated in 10% formaldehyde for pathologic analysis. Tissue specimens stained with hematoxylin & eosin were evaluated under light microscope by "Histologic Activity Index (HAI)" scoring for presence of portal inflammation, fibrosis and necrosis [10]. Rats in Group-1 and Group-2 showed slight portal inflammation (score-1) in the contact surface of liver, in Group-3 portal inflammation was moderate (score-2) in contact surface, and mild (score-1) in a distance of 1 cm from surface. None of the rats in these 3 groups showed symptoms of fibrosis or necrosis. Rats in Group-4 showed 240 ± 60 micron deep necrosis in liver contact surfaces. Compared to rats in Group-3 portal inflammation was more severe (score-3) in Group-4 and sporadic fibrosis areas had developed (Figure 3).

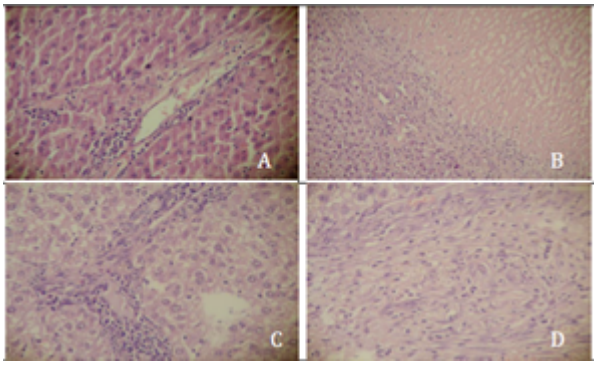

Figure 3. *Pathologic findings of liver resection specimens. A- Periportal inflammation. B- Appearance of necrosis in Group-4. C- Appearance of periportal inflammation in Grup-4. D- Appearance of fibrosis in Group-4.*

Discussion

Achievement of hemostasis in hepatic surgery continues to be one of the most crucial issues, performed either for traumatic lacerations or for benign or malignant diseases of the liver. Sinusoidal structure of the liver that enveloped in a thin capsule makes it difficult to control the bleeding originated from liver parenchyma. Uncontrollable bleedings especially seen in trauma patients is one of the most important causes of mortality [11]. Therefore quick and effective controlling of bleeding during hepatic surgery, reduces the amount of blood loss and operative complications. This also provides reduction in the need for transfusion, shorten the operation time and length of stay in hospital. In traumatic injuries of the liver, some patients are followed without surgery according to protocols whereas for some bleedings topical hemostatic agents, intrahepatic balloon tamponades, suturing and packing maybe required [12,13]. Therefore, in this study gauze tampon compress were applied for bleeding in Group-1 and hemostasis was achieved by continuous sutures in Group-2. Findings from these conventional techniques were compared with hemostasis achieved with $SoCO_2$. Today several devices and hemostatic agents are used to simplify hemostasis, to shorten hemostasis time and provide a more bloodless field for the surgeon [14-19]. In recent years use of topical hemostatic agents has increased [20]. Therefore, significant decrease is noted in morbidity and mortality rates in surgeries of the liver [21]. Some evidence from randomized controlled trials exists regarding the use of fibrin sealants [22,23] or combined with a carrier matrix [24,25]. However, the routine use of topical hemostatic agents has to be judged against noticeable additional costs. It was shown in two studies [24,26] to be superior in obtaining intraoperative hemostasis over argon plasma coagulation in liver resection by reducing the time to hemostasis significantly. Amount of bleeding, simplicity of material use, cost-benefit ratio, storing conditions are also important in addition to surgeon`s preference for choice of hemostasis method [27,28].

In the past, cryosurgery was used for different purposes such as hemostasis or tumor ablasion [29]. Especially "Argon" and

"Nitrous Oxide" were used for cryohemostasis; however, with development of ultrasonic and electromagnetic energy devices, they were not considered enough appropriate for clinical applications [11,30]. Purpose of cryosurgery is to obliterate circulation of damaged tissue by freezing, thus provide coagulation. Coagulation is formed as a result of vascular endothelium damage during freezing. However vascular structures can re-bleed secondary to liquefaction of blood clots during the melting period [31,32]. In this study, re-bleeding of relatively large vessels in the liquefaction process following freezing was also encountered. However the surgeon is able to work in a clean and bloodless field and has opportunity to ligate the re-bleeding areas during melting which can be extended with repeated $SoCO_2$ applications.

Strong cryogenicity effect of $SoCO_2$ was shown in patients who were accidentally in direct contact with $SoCO_2$. In normal conditions carbondioxide is found in gaseous state, liquefies under high pressures. It is then converted into solid state under laboratory conditions. Surface temperature of $SoCO_2$ is -78.5°C. It changes directly to gaseous state from solid state, bypassing the liquid phase (sublimation) in normal conditions. As a result it does not leave sediment on the contact tissue. Carbondioxide gas procured from several sources is purified and frozen to -20°C. And liquid CO_2 freezes itself to solid state under atmospheric pressure of 20 bars. Sublimation provides a high cooling capacity (152 Kcal/kg) in very low temperatures (-78.5°C) [33].

Kopelman et al. applied cryosurgery -160°C for 10 minute on traumatic grade III-IV wounds they have created in pig livers. They have demonstrated that cryohemostasis significantly reduced the blood loss and substantially attenuated hemorrhagic shock [11]. Likewise in our study, in the Group-3 and Group-4 in which hemostasis was achieved with $SoCO_2$, statistically significant reduced bleeding and shorten hemostasis time were established.

Garanchini et al. [34], compared bipolar vascular sealing devices and classic clamp-crush technique in liver parenchyma transection. They used transaminase values as indication of parenchymal damage which showed statistically significant increase in the group bipolar vascular sealing device was used. There was no statistically significant difference between groups in terms of the serum transaminase levels in our study.

Matsumoto et al. reported histologic outcomes during cryosurgery in the liver. According to this study tissue edema and central necrosis was detected after a ~3hrs follow up and fibrosis was detected after a ~1 week follow up after cryosurgery [35]. In our study that consistent with this article, tissue inflammation and edema was observed in the early stage in Group-3 and sporadic areas of fibrosis were observed in Group-4 that presents findings one week after $SoCO_2$ contact.

Recently, thermal energy devices were developed for hepatic resections that causing heat coagulation necrosis in the liver parenchymal. These devices produce a necrotic zone thus enables resection. Stavrou et al. utilized this technique in 28 cases and found the mean blood loss to be less than 100 ml. Disadvantages of these devices were, probability of producing thermal damage in main vessels where it is close and its use in the hilar area is also limited. It also produces 1 cm area of necrosis in the parenchyma secondary to heat and causing excessive parenchyma loss in cases requiring wide resections. Several complications, including intrahepatic or subhepatic abscess, was noted in 20% of the patients [36]. In our study, necrosis area was deteted within 240 ± 60 micron depths from the surface in Group-3 and Group-4. Cryohemostasis with $SoCO_2$ is considered to be an appropriate agent due to both creating necrosis in a limited area and do not cause further increasing in the biochemical parameters compared to the other groups. Cryohemostasis with $SoCO_2$ is considered to be an appropriate agent due to create necrosis in a limited area and do not cause differences in terms of biochemical parameters compared to the other groups. Thermal energy devices raise the tissue temperature to 70°C, resulting in reactions causing inflammation, edema and carbonization. Adhesions and increased infection problems may arise due to tissue carbonization. $SoCO_2$ assisted cryohemostasis provides hemostasis without residue on the contact surface of liver, and creating smaller necrosis area can result in decline of infection risk.

Conclusion

In conclusion, our study showed that the $SoCO_2$ assisted hemostasis decreases the blood loss, shortens the hemostasis time, enabling the surgeon to work in a bloodless surgical site. $SoCO_2$ also does not leave a residue due to its sublimation feature, easily procurable and low-cost. Although further studies are required, we believe $SoCO_2$ is an effective hemostatic agent in liver surgery.

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***Correspondence to:**

Ibrahim Ali Ozemir

Department of General Surgery

Istanbul Medeniyet University

Turkey